Appeal No. 2017-1120

United States Court of Appeals

for the

Federal Circuit

JANSSEN BIOTECH, INC., NEW YORK UNIVERSITY,

Plaintiffs-Appellants,

– v. –

CELLTRION HEALTHCARE CO., LTD., CELLTRION, INC., HOSPIRA INC.,

Defendants-Appellees.

APPEAL FROM THE UNITED STATES DISTRICT COURT FOR THE DISTRICT OF MASSACHUSETTS IN CASE NOS. 15-CV-10698 AND 16-11117, SENIOR JUDGE MARK L. WOLF

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CERTIFICATE OF INTEREST

FORM 9. Certificate of Interest **Form 9 Rev. 03/16**

UNITED STATES COURT OF APPEALS FOR THE FEDERAL CIRCUIT

Janssen Biotech, Inc. and New York University **v.** Celltrion Healthcare Co., Ltd. et al.

Case No. 2017-1120

CERTIFICATE OF INTEREST

Counsel for the:
☐ (petitioner) ☒ (appellant) ☐ (respondent) ☐ (appellee) ☐ (amicus) ☐ (name of party)

Janssen Biotech, Inc. and New York University

certifies the following (use "None" if applicable; use extra sheets if necessary):

1. Full Name of Party Represented by me	2. Name of Real Party in interest (Please only include any real party in interest NOT identified in Question 3) represented by me is:	3. Parent corporations and publicly held companies that own 10 % or more of stock in the party
Janssen Biotech, Inc.	Janssen Biotech, Inc.	Johnson & Johnson
New York University	New York University	None

4. The names of all law firms and the partners or associates that appeared for the party or amicus now represented by me in the trial court or agency or are expected to appear in this court (**and who have not or will not enter an appearance in this case**) are:

Patterson Belknap Webb & Tyler LLP (Andrew Cohen and Viviane Scott), Nutter McClennan & Fish LLP (Alison Casey and Heather Repicky)

Jan 26, 2017
Date

/s/ Gregory L. Diskant
Signature of counsel

Please Note: All questions must be answered

Gregory L. Diskant
Printed name of counsel

cc:

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ABBREVIATIONS

Parties	
Janssen	Appellants Janssen Biotech, Inc. and New York University, collectively
Celltrion	Appellees Celltrion Healthcare, Inc., Ltd., Celltrion, Inc., and Hospira, Inc., collectively
Patents	
'471 Patent	U.S. Patent No. 6,284,471
'195 Reference Patent	U.S. Patent No. 5,698,195
'272 Reference Patent	U.S. Patent No. 5,656,272
'444 Reference Patent	U.S. Patent No. 6,790,444
'093 Application	U.S. Application No. 08/192,093
'413 Parent Application	U.S. Application No. 08/013,413
'799 Application	U.S. Application No. 08/324,799
'827 Priority Application	U.S. Application No. 07/670,827
Other	
SJ Ruling	Summary judgment ruling
URAA	Uruguay Round Agreements Act

STATEMENT OF RELATED CASES

No appeal in this matter was previously before this or any other court.

A co-pending case in this Court, captioned *In re Janssen Biotech, Inc.*, No. 17-1257, would directly affect or be directly affected by the decision in this appeal. A note to the file in this case, entered on December 16, 2016, states that this appeal and Appeal No. 17-1257 "shall be considered companion cases and assigned to the same merits panel for oral argument."

Counsel for Appellants are not aware of any other case that will directly affect or be directly affected by the decision in this appeal.

JURISDICTIONAL STATEMENT

The district court had jurisdiction over this patent infringement action pursuant to 28 U.S.C. §§ 1331 and 1338(a). On September 26, 2016, the district court directed the entry of partial final judgment under Federal Rule of Civil Procedure 54(b) that the asserted claims of U.S. Patent No. 6,284,471 are invalid for obviousness-type double patenting. Appellants filed a timely notice of appeal on October 24, 2016. This Court has jurisdiction over this appeal pursuant to 28 U.S.C. §§ 1292(c) and 1295(a)(1).

INTRODUCTION

This appeal concerns Janssen's U.S. Patent No. 6,284,471 (the '471 Patent). The '471 Patent covers certain monoclonal antibodies, including the infliximab or cA2 antibody, which is useful for treating Crohn's disease, rheumatoid arthritis and other debilitating diseases. In this appeal, Janssen challenges two district court rulings granting summary judgment that claims 1, 3 and 5-7 of the '471 Patent are invalid for obviousness-type double patenting: (1) in view of U.S. Patent No. 6,790,444 (the '444 Reference Patent); and (2) in view of U.S. Patent Nos. 5,698,195 (the '195 Reference Patent) and 5,656,272 (the '272 Reference Patent). Although both rulings concerned obviousness-type double patenting, they present separate and distinct issues for this Court.

1. The *Gilead* SJ Ruling: In its motion based on the '444 Reference Patent, Celltrion argued that under *Gilead Sciences, Inc. v. Natco Pharma Ltd.*, 753 F.3d 1208 (Fed. Cir. 2014), the asserted claims of the '471 Patent are invalid in view of the later-filed but earlier-expired '444 Reference Patent. But as the district court recognized, the facts here are unlike those in *Gilead*. In *Gilead*, a patent that issued from an application filed after the effective date of the Uruguay Round Agreements Act ("URAA") (i.e., a "post-URAA" or "post-GATT" patent) was held invalid for obviousness-type double patenting in view of another post-URAA patent, where each patent had a statutory term of 20

years from its earliest priority application and the patentee had crafted separate chains of applications with different priority dates so that it would obtain more than 20 years of patent protection. The patent asserted in *Gilead* claimed priority to an application in the later chain so that it expired later. By contrast, Janssen's '471 Patent-in-suit issued from an application filed before the URAA's effective date (i.e., a "pre-URAA" or "pre-GATT" patent) and has a conventional 17-years-from-issuance statutory term. Janssen's '444 Reference Patent, which claims the same earliest priority date as the '471 Patent, was filed after the URAA's effective date and is thus a post-URAA or post-GATT patent whose term was limited to 20 years from that priority date, causing it to expire before the expiration of the '471 Patent.

Unlike the patentee in *Gilead*, Janssen did nothing to extend the term of either the '471 or '444 Patent. The only reason the '471 Patent expires later than the '444 Reference Patent is that Congress limited post-URAA patents (such as the '444 Patent) to terms of 20 years from the filing of their earliest priority application, while providing that pre-URAA patents (such as the '471 Patent) retain their traditional term of 17 years from issuance, even if that term is greater than 20 years from the filing of their earliest priority application.

This Court has never relied on obviousness-type double patenting based on a later-filed, earlier-expiring post-URAA patent to invalidate a pre-

URAA patent with a 17-years-from-issuance term based obviousness-type double patenting. The district court's decision, extending *Gilead* to this situation, is at odds with Congress's decision to retain the traditional 17-years-from-issuance term for pre-URAA patents and is at odds with settled law on obviousness-type double patenting for pre-URAA patents.

2. The Safe Harbor SJ Ruling: Celltrion's motion based on the '195 and '272 Reference Patents raised issues that also were then-pending before the PTAB on Janssen's appeal from a final rejection in a reexamination that Celltrion had requested. The district court rejected Janssen's request to defer consideration of that motion pending a final ruling in the reexamination, and it granted summary judgment that the asserted claims of the later-issued '471 Patent are invalid for obviousness-type double patenting in view of the earlier-issued '195 and '272 Patents. In reaching that result, the district court committed three legal errors:

a) The district court erred in ruling that the safe harbor provided by 35 U.S.C. § 121 does not apply because the application for the '471 Patent initially was denominated a continuation-in-part ("CIP") rather than a divisional, when: (i) the application was filed in response to a restriction requirement and was prosecuted as a divisional, (ii) the applicant never sought or obtained any

claims based on the disclosure first added in the CIP; (iii) during prosecution, the applicant and the Examiner both reasonably relied on the safe harbor's applicability, and (iv) the issued claims of the '471 Patent are fully supported by the original disclosure of its parent application and fully consonant with the restriction that forced its separate filing.

b) The district court also erred in ruling that the applicable test for obviousness-type double patenting here is the one-way test, rather than the appropriate two-way test, based on a novel theory that the two-way test does not apply where the applications in question were filed on the same day. The rationale for the two-way test does not require the applications to have been filed on different days, and the application for the '471 Patent was filed earlier by serial number, albeit on the same day as the application for the '272 Reference Patent.

c) The district court also erred in holding that the asserted claims of the '471 Patent would be invalid even under the two-way test, based on an incorrect comparison between the *claims* of the '195 and '272 Patents and the *specification* of the '471 Patent. The correct analysis compares the *claims* of each patent to the *claims*

of the other. Celltrion provided no evidence under the correct analysis and was not entitled to summary judgment.

In its co-pending appeal, No. 17-1257, Janssen appeals from a decision by the Patent Trial and Appeals Board affirming a final rejection in reexamination of claims 1-7 of the '471 Patent as invalid for obviousness-type double patenting in view of the '195 and '272 Reference Patents. Because the '471 Patent was amended in reexamination, a decision favorable to Janssen in the co-pending appeal would supersede the district court's ruling, which was based on the un-amended form of the '471 Patent. In particular, a decision in Janssen's favor in the co-pending appeal on the applicability of the statutory safe harbor would require reversal of the district court's ruling on invalidity for obviousness-type double patenting in view of the '195 and '272 Reference Patents, leaving the ruling of invalidity in view of the '444 Reference Patent as the only other issue to be resolved in this appeal. Conversely, a decision in the co-pending appeal upholding the final rejection of the '471 Patent would render this appeal moot. These considerations weigh in favor of this Court deciding Appeal No. 17-1257 before addressing the issues here.

STATEMENT OF ISSUES

1. The *Gilead* SJ Ruling: In granting summary judgment that the asserted claims of the pre-URAA '471 Patent are invalid for obviousness-type double patenting in view of the post-URAA '444 Reference Patent, did the district court err where the only reason the '471 Patent has a longer term than the '444 Patent is that Congress decided that pre-URAA patents should retain their traditional terms of 17 years from issuance, if that term extends longer than 20 years from filing?

2. The Safe Harbor SJ Ruling: In granting summary judgment that the asserted claims of the '471 Patent are invalid for obviousness-type double patenting in view of the '272 and '195 Reference Patents, did the district court err: (a) in holding that the statutory safe harbor is inapplicable because the application for the '471 Patent was originally denominated as a CIP rather than a divisional, where the application for the '471 Patent was filed and prosecuted in response to a restriction requirement, its issued claims are fully supported by the original disclosure of the parent application and consonant with a non-elected group of the restriction requirement, and where the application has been amended to be formally designated as a divisional and to conform to the disclosure of the parent application in which the restriction was applied; (b) in holding that the same-day filing of the applications for the '471 and '272 Patents requires application of the

one-way test for obviousness-type double patenting rather than the two-way test, when the rationale for the two-way test does not require filing on different days and the application for the '471 Patent was filed earlier by serial number; and (c) in holding that summary judgment would be warranted even under the two-way test based on an incorrect comparison between the claims of the '195 and '272 Patents and the *specification* of the '471 Patent, when Celltrion offered no evidence on the correct comparison between the *claims* of the various patents to each other?

STATEMENT OF THE CASE

Janssen alleges, *inter alia*, that Celltrion infringed claims 1, 3 and 5-7 of Janssen's '471 Patent by filing an abbreviated Biologic License Application for a drug that is biosimilar to REMICADE®, Janssen's commercial embodiment of the '471 Patent.

The district court granted Celltrion's motions for partial summary judgment that the asserted claims of the '471 Patent are invalid for obviousness-type double patenting. The district court then granted Celltrion's motion under Rule 54(b) of the Federal Rules of Civil Procedure for entry of partial final judgment that the asserted claims of the '471 Patent are invalid.

STATEMENT OF FACTS

A. The '471 Patent

The '471 Patent is entitled "Anti-TNFA Antibodies and Assays Employing Anti-TNFA Antibodies." Its claims cover certain antibodies that can bind to and neutralize a substance in the human body known as tumor necrosis factor alpha or TNFα. The claimed antibodies include an antibody known as cA2 or infliximab, which is useful in treating Crohn's disease, rheumatoid arthritis and other chronic diseases. Appx111-197.

The '093 Application, which issued as the '471 Patent, was filed on February 4, 1994. It claims priority to the '827 Priority Application, which was filed on March 18, 1991. The '471 Patent issued on September 4, 2001 and expires on September 4, 2018, 17 years from its issue date.

The claims of the '471 Patent are directed to chimeric antibodies "capable of binding with an epitope specific for human tumor necrosis factor TNFα." Claim 1 is a representative claim. It recites:

> A chimeric antibody comprising at least a part of a human immunoglobulin constant region and at least part of a non-human immunoglobulin variable region, said antibody capable of binding with an epitope specific for human tumor necrosis factor TNFα, wherein the non-human immunoglobulin variable region comprises an amino acid sequence selected from the group consisting of SEQ ID. NO: 3 and SEQ ID NO: 5.

Appx197. The claims of the '471 Patent do not claim administering antibodies to treat any particular disease.

B. The '471 Invention Provides Important Clinical Benefits and Has Enjoyed Commercial Success

Janssen's commercial embodiment of the '471 Patent, the biologic drug REMICADE®, has been approved by the FDA for treating Crohn's disease, rheumatoid arthritis and other diseases. Because of its clinical benefits, REMICADE® has achieved tremendous commercial success, with U.S. sales averaging nearly $4 billion annually. This extraordinary level of sales reflects the importance of the '471 invention.

C. Celltrion's Summary Judgment Motions

Celltrion filed two motions for summary judgment, raising two different theories that the asserted claims of the '471 Patent are invalid for obviousness-type double patenting.

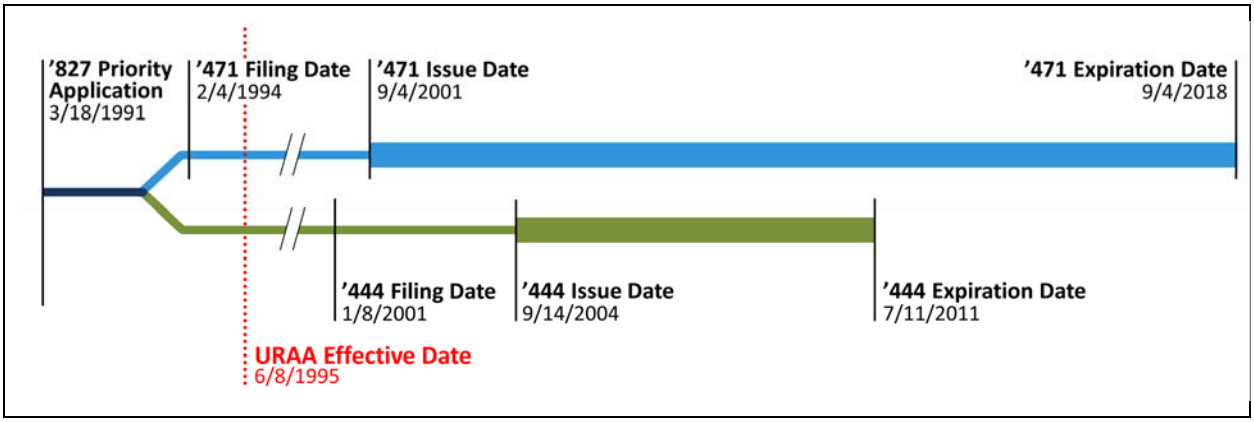
1. The *Gilead* Motion

In the so-called "*Gilead* Motion," Celltrion argued that *Gilead v. Natco*, 753 F.3d 1208, requires summary judgment that the asserted claims of the '471 Patent are invalid for obviousness-type double patenting in view of the '444 Reference Patent.

a. Facts Relevant to the *Gilead* Motion

As a pre-URAA patent that issued from an application filed before the URAA's effective date, the '471 Patent has a statutory term of 17 years from issuance. In contrast, the '444 Reference Patent, as a post-URAA patent that issued from an application filed after the URAA's effective date, has a statutory term of 20 years from its earliest priority application. Both patents claim priority to the same earliest priority application, the '827 Priority Application. Appx111; Appx198. Janssen did not do anything to extend the term of either patent.

The relevant dates for the '471 and '444 Patents are shown below:



Celltrion argued that *Gilead*, 753 F.3d 1208, is "indistinguishable" from the present case. Appx4484. But *Gilead*—unlike this case—involved two post-URAA patents with statutory terms of 20 years from the filing of two different priority applications with two different priority dates. In *Gilead*—unlike here—the patentee had crafted different chains of applications with different priority dates, with the patent asserted in litigation claiming priority to an

application in the later chain so that it would expire later. This Court "conclude[d] under the circumstances of th[at]case" that the later-issued Gilead patent could qualify as a double-patenting reference for the post-URAA patent-in-suit. 753 F.3d at 1212.

The *Gilead* Motion presented the issue of whether a pre-URAA patent should be held invalid for obviousness-type double patenting in view of a later-filed but earlier-expiring post-URAA patent, where the only reason the pre-URAA patent-in-suit expires later than the post-URAA reference patent is that Congress chose to treat pre- and post-URAA patents differently. *See* 35 U.S.C. §§ 154(a)(2) and (c)(1).[1]

b. The *Gilead* SJ Ruling

In its ruling on the *Gilead* Motion, the district court rejected Celltrion's assertion that this case is indistinguishable from *Gilead*, Appx4484, but nonetheless granted Celltrion's motion for summary judgment that the asserted claims of the pre-URAA '471 Patent are invalid for obviousness-type double patenting in view of the post-URAA '444 Reference Patent. Appx672-680; Appx2-3. The district court stated that this case is "factually different than *Gilead*" so that *Gilead* does not control the outcome here, Appx675, but it nonetheless

[1] For purposes of the *Gilead* Motion, Janssen did not dispute that the claims of the '471 and '444 Patents are not patentably distinct.

extended *Gilead* to the hybrid situation presented by this case. In doing so, the district court relied heavily on *Gilead*'s statement that "[i]t is a bedrock principle of our patent system that when a patent expires, the public is free to use not only the same invention claimed in the expired patent but also obvious or patentably indistinct modifications of that invention." Appx675-676 (quoting *Gilead*, 753 F.3d at 1214). The district court described *Gilead* as broadly holding "that a later-issuing, earlier expiring patent can act as a double-patenting reference for an earlier-issuing, later-expiring patent," Appx2, even though this Court expressly and repeatedly limited *Gilead*'s holding to the "*circumstances of th[at] case*," which, as the district court found, are unlike the circumstances here. *Gilead*, 753 F.3d at 1212 (emphasis added); *see also id.* at 1217 (same).

The district court (correctly) stated that in enacting the URAA, Congress "[did] not intend[] to alter the judicial doctrine of obviousness double patenting …." Appx3 (citing *Gilead*, 753 F.3d at 1216). However, the district court's decision departs from the well-established rule for pre-URAA patents that "'[a]ll proper double patenting rejections … rest on the fact that a patent has been issued and later issuance of a second patent will continue [patent] protection, beyond the expiration of the first patent' on the same invention or an obvious variation thereof," *Sun Pharm. Indus., Ltd. v. Eli Lilly & Co.*, 611 F.3d 1381, 1389 (Fed. Cir. 2010) (quoting *In re Kaplan*, 789 F.2d 1574, 1579-80 (Fed. Cir. 1986)),

and is at odds with settled law that obviousness-type double patenting only applies where there has been an "improper" or "unjustified" extension of the patent term. *In re Braat*, 937 F.2d 589, 592 (Fed. Cir. 1991). The district court did not identify any improper conduct by Janssen, and indeed there was none.

2. The Reexam Motion

In the so-called "Reexam Motion," Celltrion moved for summary judgment that the asserted claims of the '471 Patent are invalid for obviousness-type double patenting in view of the '272 and '195 Reference Patents. That motion raised issues that were then-pending before the PTAB on Janssen's appeal from a final rejection in reexamination of claims 1-7 of the '471 Patent.

a. The Restriction Requirement in the '413 Parent Application

The '471 Patent-in-suit and the '272 and '195 Reference Patents all descend from the '413 Parent Application, which was filed on February 2, 1993. The '413 Parent Application pertained to antibodies for TNFα and methods for treatment using a pharmaceutical composition containing an antibody.

During prosecution of the '413 Parent Application, the PTO issued a five-way restriction requirement, identifying five patentably distinct inventions. The restriction identified Group I as claims "drawn to monoclonal antibodies, … chimeric antibodies, pharmaceutical compositions, and assay methods," and

identified Group IV as claims drawn to methods of treatment using a pharmaceutical composition that contains an antibody. Appx1808-1839 at Appx1810.

In the '413 Parent Application, Janssen elected the Group IV method-of-treatment claims for examination, and the Group I antibody claims were withdrawn from further consideration. Appx4106-4116 at Appx4107-4108; Appx4207-4213.

b. The '272 and '195 Reference Patents

Janssen eventually abandoned the '413 Parent Application, but continued to pursue examination of the Group IV method-of-treatment claims in continuing applications that issued as the '272 and '195 Reference Patents. The application that issued as the '272 Patent was filed on February 4, 1994 and issued on August 12, 1997. Appx294. Claim 7 of the '272 Patent is directed to methods of treating Crohn's disease by administering the cA2 antibody. Appx380. The application that issued as the '195 Patent was filed on October 18, 1994, as a continuation-in-part of three earlier applications, including the application that issued as the '471 Patent. Appx381; Appx419. The '195 Patent issued on December 16, 1997. Appx381. Reference claim 6 of the '195 Patent is directed to methods of treating rheumatoid arthritis by administering the cA2 antibody. Appx472. As patents issuing from pre-URAA applications, the '272 and '195

Patents each expired 17 years from their issuance, on August 12, 2014 and December 16, 2014, respectively.

c. Prosecution of the Application that Issued as the '471 Patent-in-Suit

The '093 Application, which issued as the '471 Patent, was filed in response to the restriction requirement in the '413 Parent Application. Janssen used the '093 Application to pursue claims directed to a non-elected invention, i.e., antibody claims in non-elected Group I. Appx4107-4109; Appx4254-4255 at Appx4254.

The '093 Application was filed on February 4, 1994, the same day as the application that issued as the '272 Patent and eight months before the filing of the application that issued as the '195 Patent. By serial number, the '093 Application was filed earlier than the application for the '272 Patent, that is, it has U.S. Application No. 08/192,093, compared to U.S. Application No. 08/192,102, assigned to the application for the '272 Patent. Appx111, Appx294. The PTO took much longer to examine the application for the '471 Patent than the applications for the '195 and '272 Patents. The '471 Patent issued on September 4, 2001, more than three years after the '272 and '195 Patents issued.

The '093 Application initially was designated as a CIP application because it contained supplemental disclosures not present in the '413 Parent

Application. Appx4112. However, Janssen never sought claims directed at the supplemental disclosure and that disclosure was not needed to support any claim in the '093 Application. During prosecution, all of the claims of the '093 Application were amended to be consonant with restriction Group I, so that the Application was, in substance, a divisional for purposes of its claims. Appx3777-3793 at Appx3789-3790. Janssen expressly advised the PTO that it was pursuing the '093 Application "pursuant to the restriction requirement set forth in [a] parent application." Appx3934-3941 at Appx3938; *see also* Appx3967-3992 at Appx3980.

During the original examination of the '093 Application, the Examiner provisionally rejected certain then-pending claims for obviousness-type double patenting in view of Group I antibody claims in the co-pending '799 Application, which later issued as the '195 Patent. Appx3943-3959 at Appx3945-3946; Appx4109. Janssen then submitted a preliminary amendment in the '799 Application, cancelling the Group I antibody claims in that application and leaving only Group IV claims directed to methods of treating rheumatoid arthritis. Appx4288-4292; Appx4110.

In a subsequent office action, the Examiner nonetheless maintained the obviousness-type double patenting rejection in the '093 Application. In response, Janssen cited the restriction requirement in the '413 Parent Application

and argued that the '093 Application was entitled to the benefit of the statutory safe harbor:

> [T]he claims now pending in Serial No. 08/324,799 no longer correspond to Claims 1-28, 33-40, 59-61 and 63 [of the '093 Application]. *35 U.S.C. § 121 precludes an obviousness-type double patenting rejection in this case. See the restriction requirement … in U.S. Serial No. 08/013,413 …. Withdrawal of the rejection is respectfully requested*.

Appx4307 (emphasis added). The Examiner then agreed with Janssen and withdrew the double-patenting rejection. Appx4321-4328 at Appx4322; Appx4110-4113.

It was reasonable for Janssen and the Examiner to believe that the '093 Application was entitled to the benefit of the safe harbor. As explained in the Declaration of Janssen's expert Stephen Kunin, former Deputy PTO Commissioner for Patent Examination Policy, patent practitioners and the PTO understood at the time that CIP applications were entitled to the statutory safe harbor if they were filed in response to a restriction requirement and otherwise complied with the requirements of § 121, as was the case with the '093 Application. Appx3789-3792; Appx4112.

Janssen reasonably relied on the Examiner's decision to withdraw the provisional double patenting rejection after Janssen invoked the statutory safe harbor. If the Examiner had maintained the rejection, Janssen could easily have

amended the '093 Application to delete the supplemental material since it was not needed to support any claims and could have amended the CIP designation to re-designate the '093 Application as a divisional. Appx4112-4113; Appx3791-3792. The Examiner's withdrawal of the provisional double patenting rejection made these steps unnecessary.

The issued claims of the '471 Patent, directed to antibodies capable of binding with an epitope specific for human necrosis factor TNFα, are consonant with the restriction and fully supported by the original disclosure of the '413 Parent Application. The '471 claims are not directed to using antibodies to treat particular diseases.

d. Reexamination of the '471 Patent

In 2013, the PTO instituted an *ex parte* reexamination of the '471 Patent, at Celltrion's then-anonymous request. During the reexamination, the PTO permitted Janssen to file amendments: (a) deleting the new material in the '471 Patent to substantively conform its disclosure to that of the '413 Parent Application, and (b) expressly designating the '471 Patent as a divisional. Appx4097-4105 at Appx4104-4105; Appx3792.

After Janssen filed its notice of appeal in this case, the PTAB affirmed a final rejection in reexamination of claims 1-7 for obviousness-type double

patenting in view of the '195 and '272 Reference Patents. Janssen's appeal from the PTAB's decision is co-pending in this Court as Appeal No. 17-1257.

3. The Safe Harbor SJ Ruling

The district court rejected Janssen's assertion that a decision on the Reexam Motion should be deferred pending a final determination in the reexamination, and granted summary judgment on the Reexam Motion. Appx791-811; Appx3-6; Appx20-43.

The safe harbor issue: In the district court, Janssen asserted that the safe harbor of 35 U.S.C. § 121 was applicable and required denial of the Reexam Motion.[2] The district court erroneously held that the safe harbor was inapplicable because the '093 Application initially was denominated as a CIP, rather than as a divisional. Appx4, *see also* Appx28-33; Appx40-41. The district court reached its conclusion even though: (i) the '093 Application was filed in response to a restriction requirement and was prosecuted as a divisional; (ii) none of the claims in the '093 Application claimed or required the support of any disclosure not also found in the '413 Parent Application; (iii) all of the issued claims of the '471

[2] Section 121 provides in pertinent part that a patent issuing on an application filed as a result of a restriction requirement "shall not be used as a reference … against a divisional application or against the original application or any patent issued on either of them, if the divisional application is filed before the issuance of the patent on the other application." 35 U.S.C. § 121.

Patent are consonant with non-elected Group I of the restriction applied in the '413 Parent Application; and (iv) the '471 Patent was amended in reexamination with the PTO's approval, to delete all added disclosure and substantively conform it to that of the '413 Parent Application, and to designate the '471 Patent as a divisional.

Applicability of the two-way test: Where the safe harbor does not apply, this Court applies two different tests for obviousness-type double patenting depending on the facts of the particular case: the one-way test or the two-way test. Under the one-way test, the issue is whether a challenged claim would have been obvious in view of a claim in a reference patent. *In re Berg*, 140 F.3d 1428, 1432 (Fed. Cir. 1998). Under the two-way test, the issues are whether: (1) the challenged claim would have been obvious in view of the reference claim; and (2) vice versa. *Id*.

The district court erroneously held that the one-way test applies on the facts of this case.[3] Appx4-5; Appx801-806; Appx33-38. In addressing this issue, the district court recognized that the two-way test applies "where 'the PTO is solely responsible for the delay in causing [a] second-filed application to issue

[3] Janssen did not dispute that the asserted claims of the '471 Patent would be obvious in light of the '195 and '272 Patents if the one-way test for obviousness-type double patenting were applied. Appx5.

prior to the first.'" Appx4-5 (quoting *In re Hubbell*, 709 F.3d 1140, 1149 (Fed. Cir. 2013)); *see also* Appx803. The district court also stated (correctly) that "there may be a genuine dispute of fact concerning whether the PTO is solely responsible for the '471 Patent issuing after the '195 and '272 Patents." Appx5; *see also* Appx38.

For purposes of Celltrion's summary judgment motion, the latter determination should have ended the inquiry and required a trial on the application of the two-way test. The district court, however, erroneously held that the two-way test is inapplicable because "the application for the '471 Patent was filed on the same day as the application for the '272 Patent." Appx5. In the district court's view, "[t]he PTO did not decide the applications [for the '471 and '272 Patents] in the reverse order of filing" because they were filed on the same day, and "[t]herefore, the two-way test is not applicable." *Id.*; *see also* Appx35; Appx803-804.

The district court did not, and could not, cite any decision holding that filing on separate days is a prerequisite for application of the two-way test. The district court also did not consider the actual order in which the applications for the '471 and '272 Patents were filed. Had the court considered that issue, it would have seen that the application for the '471 Patent was filed before the applications for the '272 and '195 Reference Patents, as reflected by their serial numbers, and

that the applications were decided in the reverse order of filing, with the '272 and '195 Patents issuing in 1997 and the '471 Patent issuing in 2001.

On these facts, and in view of the district court's determination that there are disputed fact issues on whether the PTO was solely responsible for the '471 Patent issuing after the '272 and '195 Reference Patents, it was error for the district court to hold on summary judgment that the one-way test applies here.

Application of the two-way test: The district court committed further error when it stated that "the Asserted Claims would also be obvious in light of the '195 Patent and the '272 Patent" even if the two-way test were applied. Appx5; *see also* Appx38-42. The district court reached that conclusion by applying a legally incorrect analysis for obviousness-type double patenting.

Obviousness-type double patenting involves a claim-to-claim comparison, between the *claims* in one patent and the *claims* of another patent.[4] But instead of considering whether the *claims* of the '471 Patent rendered the claims of the '272 and '195 Patents obvious, the district court incorrectly addressed a different issue: whether the *specification* of the '471 Patent rendered the '272 and '195 claims obvious. Appx39-42; Appx808. Using that incorrect comparison,

[4] *See Gen. Foods Corp. v. Studiengesellschaft Kohle mbH*, 972 F.2d 1272, 1277 (Fed. Cir. 1992).

the district court concluded that the '471 *specification* would have rendered the '195 and '272 claims obvious because the '471 *specification* "discloses the same uses for [the] infliximab [antibody] that are claimed in the '195 and '272 Patents: treatment of Crohn's disease and rheumatoid arthritis." Appx6; *see also* Appx40-42.

Celltrion did not address the correct claim-to-claim comparison, between the *claims* of the '471 Patent and the '195 and '272 claims. As noted above, the claims of the '471 Patent are directed to certain chimeric antibodies and—unlike the '195 and '272 claims—not to methods of using a particular antibody to treat any specific disease.

4. Entry of Partial Final Judgment Under Rule 54(b)

After granting summary judgment for Celltrion on both motions, the district court granted Celltrion's motion under Rule 54(b) of the Federal Rules of Civil Procedure for partial final judgment that the asserted claims of the '471 Patent are invalid for obviousness-type double patenting. Appx44-61. This appeal followed.

SUMMARY OF ARGUMENT

1. The *Gilead* SJ Ruling: The district court erred in holding that the pre-URAA '471 Patent (which has a statutory term of 17 years from issuance) is invalid for obviousness-type double patenting in view of the post-URAA '444 Patent (which had a statutory term of 20 years from the filing of its earliest priority application), where both patents descend from the same earliest priority application and thus share the same earliest priority date, and the only reason the '471 Patent has a longer term is that Congress decided, in 35 U.S.C. § 154(c)(1), that pre-URAA patents should retain their traditional term of 17 years from issuance where that is longer than 20 years from the filing of the earliest priority application. The district court's invalidity ruling on these facts is at odds with Congress' intent and well-settled law on obviousness-type double patenting for pre-URAA patents.

2. The Safe Harbor SJ Ruling: The district court erred in granting summary judgment that the asserted claims of the '471 Patent are invalid for obviousness-type double patenting in view of the '272 and '195 Patents.

a. The district court erred in holding that undisputed facts warrant summary judgment that the § 121 safe harbor is inapplicable because the application for the '471 initially was denominated as a CIP when: (i) Janssen filed the application for the '471 Patent in response to a restriction requirement; (ii) the claims of the '471 Patent are fully supported by the original disclosure of the '413

Parent Application and consonant with non-elected Group I of the restriction applied in the '413 Parent Application; (iii) Janssen did not seek any claims based on newly added matter, so that the application for the '471 Patent was a divisional for purposes of its claims; (iv) during the original prosecution, Janssen and the PTO reasonably treated the application leading to the '471 Patent as a divisional, with the PTO accepting Janssen's argument that it was entitled to the § 121 safe harbor and withdrawing its double-patenting rejection on that basis; and (v) the '471 Patent was amended in reexamination to delete the newly added disclosure and to designate the patent as a divisional, so that it stands before this Court as a divisional in form as well as in substance.

b. The district court also erred in holding that the one-way test for double patenting is applicable, rather than the two-way test. The district court's finding that "there may be a genuine dispute of fact concerning whether the PTO is solely responsible for the '471 Patent issuing after the '195 and '272 Patents," Appx5, meant that the court should have assumed that the two-way test was applicable for purposes of deciding Celltrion's summary judgment motion. But to the contrary, the district court held that the one-way test is applicable because "the application for the '471 Patent was filed on the same day as the application for the '272 Patent." *Id*. That reasoning is not supported by any decision by this Court and is incorrect.

The two way test is intended to prevent rejections for obviousness-type double patenting of a later-issued patent if that patent issued later through no fault of the applicants, and the claims of the first-issued patent would not have been obvious in view of the later-issued patent. While this situation normally arises with applications filed on different days, same-day filing of related applications that could not properly be filed as a single application is not materially different and should not disqualify an application from the use of the two-way test. In a perfect world, such related applications, when filed on the same day, would be examined at the same speed and issue the same day. When that does not occur through no fault of the applicant, double patenting should not be available to invalidate otherwise proper patent protection.

The fact that the applications leading to the '471 and '272 Patents could not have been filed as a single application (due to the restriction in their parent application) and were filed on the same day strengthens, not weakens, the reasons for applying the two-way test. Neither application qualifies as prior art to the other, and it would be unfair to limit the protection accorded the later-issuing patent where there are fact disputes on whether its later issuance results solely from the PTO's conduct, and the claims of the earlier-issued patent are not obvious in view of the claims of the later-issued patent.

Even if this Court were to conclude that filing order governs, reversal would still be appropriate because: (1) by serial number, the application for the '471 Patent was filed before the applications for the '272 and '195 Patents, meaning that the PTO decided the applications in the reverse order of filing, and (2) as the district court recognized, "there may be a genuine dispute of fact concerning whether the PTO is solely responsible for the '471 Patent issuing after the '195 and '272 Patents." Appx5. On these facts, it was error on summary judgment to apply the one-way test.

c. The district court also erred in stating that the '471 claims would be invalid under the two-way test. The district court based that conclusion on an erroneous comparison between the claims of the '195 and '272 Patents and the *specification* of the '471 patent, when the correct comparison is a claim-to-claim comparison, between the claims of the '195 and '272 Patents and the *claims* of the '471 Patent. The asserted claims of the '471 Patent are directed to certain chimeric antibodies and do not make obvious a method of treating any disease, whereas claim 6 of the '195 Patent and claim 7 of the '272 Patent pertain to methods of using cA2 antibodies to treat rheumatoid arthritis and Crohn's disease, respectively. Under the correct claim-to-claim comparison, there is no evidence that the '471 antibody claims themselves would have made the '195 or '272 treatment methods obvious.

STANDARD OF REVIEW

The issues on this appeal are reviewed *de novo*. "Obviousness-type double patenting is a question of law that [this Court] review[s] *de novo*," *Hubbell*, 709 F.3d at 1145, "without deference." *Sun Pharm. Indus., Ltd. v. Eli Lilly & Co.*, 611 F.3d 1381, 1384 (Fed. Cir. 2010). The availability of the § 121 safe harbor is reviewed as a "question of law and without deference." *St. Jude Med., Inc. v. Access Closure, Inc.*, 729 F.3d 1369, 1379 (Fed. Cir. 2013). "[W]hether a one-way or two-way analysis applies is a question of law that [this Court] review[s] without deference." *In re Basell Poliolefine Italia S.P.A.*, 547 F.3d 1371, 1375 (Fed. Cir. 2008).

These legal questions are "based on underlying facts," *Prometheus Labs., Inc. v. Roxane Labs., Inc.*, 805 F.3d 1092, 1097 (Fed. Cir. 2015), and summary judgment is only appropriate if, "viewing the evidence in the light most favorable to the non-moving party, the movant shows that there is no genuine dispute as to any material fact and the movant is entitled to judgment as a matter of law." *MRC Innovations, Inc. v. Hunter Mfg., LLP*, 747 F.3d 1326, 1331 (Fed. Cir. 2014); *see also Anderson v. Liberty Lobby, Inc.*, 477 U.S. 242, 247-48 (1986). "This court reviews summary judgment decisions under the law of the regional circuit," and "[t]he First Circuit reviews such decisions *de novo*." *Momenta Pharms., Inc. v. Teva Pharms. USA, Inc.*, 809 F.3d 610, 614-15 (Fed. Cir. 2015).

ARGUMENT

THE DISTRICT COURT ERRED IN GRANTING SUMMARY JUDGMENT THAT THE ASSERTED CLAIMS OF THE '471 PATENT ARE INVALID FOR OBVIOUSNESS-TYPE DOUBLE PATENTING

I. The Law on Obviousness-Type Double Patenting

Obviousness-type double patenting is a "judicially-created doctrine" that guards against "unjustified… extension[s]" of the patent grant for an invention that is not "patentably distinct" from the invention described in another commonly owned patent. *Eli Lilly & Co. v. Barr Labs., Inc.*, 251 F.3d 955, 967-68 (Fed. Cir. 2001). "There are two justifications for obviousness-type double patenting." *Hubbell*, 709 F.3d at 1145. The first rationale, and "'[t]he fundamental reason for the rule of [obviousness type double patenting],'" *Boehringer Ingelheim International GmbH v. Barr Laboratories, Inc.*, 592 F.3d 1340, 1348 (Fed. Cir. 2010), is "'to prevent unjustified timewise extension of the right to exclude granted by a patent no matter how the extension is brought about.'" *Hubbell*, 709 F.3d at 1145 (quoting *In re Van Ornum*, 686 F.2d 937, 943-44 (C.C.P.A. 1982)). "The second rationale is to prevent multiple infringement suits by different assignees asserting essentially the same invention." *Id*.

Where obviousness-type double patenting is applicable, the inquiry has two steps:

> First, the court "construes the claim[s] in the earlier patent and the claim[s] in the later patent and determines the differences." Second, the court "determines whether those differences render the claims patentably distinct."

Sun, 611 F.3d at 1385 (alteration in original) (quoting *Pfizer, Inc. v. Teva Pharms. USA, Inc.*, 518 F.3d 1353, 1363 (Fed. Cir. 2008)). "'A later claim that is not patentably distinct from,' i.e., 'is obvious over[ ] or anticipated by,' an earlier claim is invalid for obviousness-type double patenting." *Id.* (quoting *Eli Lilly*, 251 F.3d at 968).

A party asserting invalidity based on obviousness-type double patenting is "required to prove double patenting by clear and convincing evidence, a heavy and unshifting burden," *Symbol Techs., Inc. v. Opticon, Inc.*, 935 F.2d 1569, 1580 (Fed. Cir. 1991), and bears the same burden for the underlying facts. *Amgen, Inc. v. F. Hoffmann-La Roche, Ltd.*, 580 F.3d 1340, 1362 (Fed. Cir. 2009).

II. In the *Gilead* SJ Ruling, the District Court Erred in Holding that the Pre-URAA '471 Patent for Obviousness-Type Double Patenting Is Invalid in View of the Post-URAA '444 Patent

The *Gilead* SJ Ruling raises an important issue of first impression in this Court: Whether a pre-URAA patent that has the traditional term of 17 years from issuance can be invalid for obviousness-type double patenting in view of a later-filed post-URAA patent that expired earlier because its term was limited under the URAA to 20 years from filing of its earliest priority application, where

both patents share the same earliest priority application (and earliest priority date) and the only reason the pre-URAA patent has a longer term is that Congress decided, in 35 U.S.C § 154(c)(1), that pre-URAA patents should retain the traditional term of 17 years from issuance even if that term is longer than 20 years from filing of the earliest priority application.

The district court's ruling that the pre-URAA '471 Patent is invalid in view of the post-URAA '444 Patent is at odds with Congress' judgment as reflected in § 154(c)(1) and at odds with settled law on obviousness-type double patenting for pre-URAA patents.

A. Obviousness-Type Double Patenting for Pre-URAA Patents

Before the URAA was enacted, "U.S. patents had an expiration date under 35 U.S.C. § 154 measured as 17 years from the date the patent issued," subject to exceptions not relevant here. *Merck & Co. v. Kessler*, 80 F.3d 1543, 1547 (Fed. Cir. 1996).

With pre-URAA patents, obviousness-type double patenting serves as "an important check on improper extensions of patent rights through the use of divisional and continuation applications …." *Boehringer*, 592 F.3d at 1346. The concern is that a patentee could file continuation applications on inventions that are not patentably distinct, with the patents issuing on different dates, creating sequential terms of 17-years from issuance so that a later-issuing patent effectively

extends the first patent's 17-year term. For pre-URAA patents, the rule against double patenting "prevent[s] an inventor from effectively extending the term of exclusivity by the subsequent patenting of variations that are not patentably distinct from the first-patented invention." *Applied Materials, Inc. v. Advanced Semi. Materials Am., Inc.*, 98 F.3d 1563, 1568 (Fed. Cir. 1996). This purpose is implicated only where a second patent continues the period of exclusivity beyond the first patent's expiration. That concern is not present here because the first-issuing '471 Patent expires last, 17 years after its issuance.

The law on double patenting for pre-URAA patents is well settled:

> "[A]ll proper double patenting rejections … rest on the fact that a patent has been issued and *later issuance of a second patent will continue [patent] protection, beyond the expiration of the first patent*" on the same invention or an obvious variation thereof.

Sun, 611 F.3d at 1389 (quoting *In re Kaplan*, 789 F.2d 1574, 1579-80 (Fed. Cir. 1986)) (emphasis added).

B. The URAA and Its Effect on Patent Expiration

1. The Term of Post-URAA Patents

The URAA was intended "to harmonize the term provision of United States patent law with that of our leading trad[e] partners which grant a patent term of 20 years from the date of filing of the patent application." *Merck*, 80 F.3d at 1547. To accomplish that purpose, Congress provided in 35 U.S.C. § 154(a)(2)

that patents issuing from applications filed after the URAA's effective date have a term of 20 years from the filing of the application for the patent or its earliest priority application. Section 154(a)(2) provides as follows:

> (2) *Term*. —…. [The] grant [of a patent] shall be for a term beginning on the date on which the patent issues and ending 20 years from the date on which the application for the patent was filed in the United States or, if the application contains a specific reference to an earlier application or applications under section 120, 121, 365(c), or 386(c), from the date on which the earliest such application was filed.

The URAA became effective on June 8, 1995.

2. The Term of Patents Resulting From Applications Filed Before June 8, 1995

The URAA's change in patent terms was a major shift in U.S. patent law. To avoid upsetting the settled expectations of parties who had applied for patents before the URAA's effective date, the URAA included "a transitional provision [that] preserves a guaranteed 17-year term," as under prior law, for patents issuing from applications filed before June 8, 1995, "if [that term] is longer than 20 years from filing …." *Merck*, 80 F.3d at 1547. The relevant provision of the URAA, 35 U.S.C. § 154(c)(1), provides as follows:

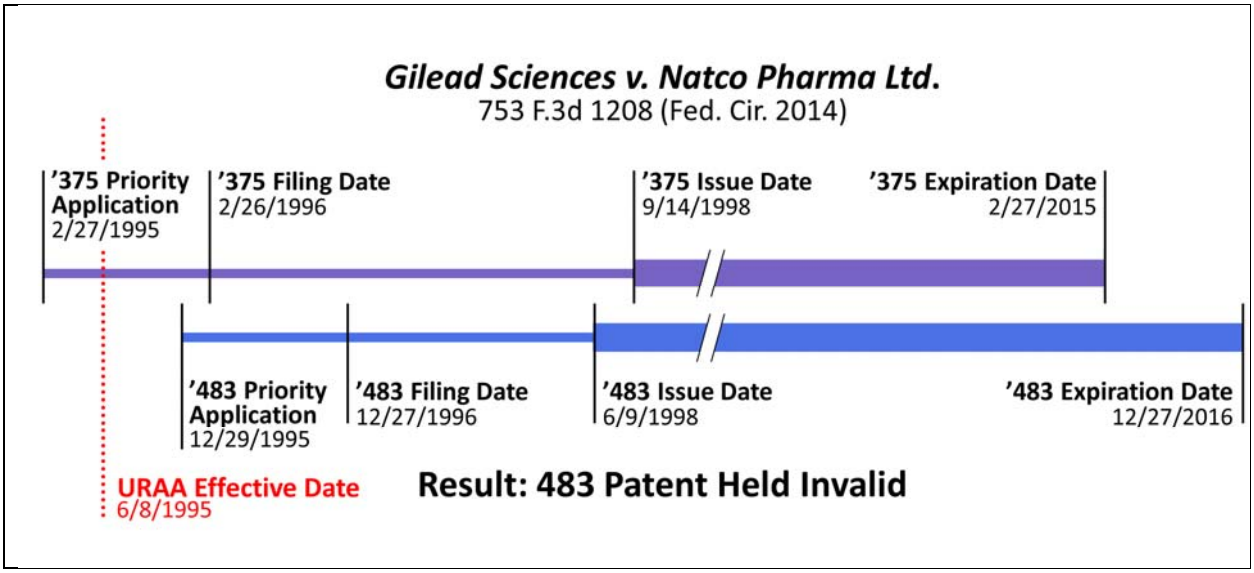
> (1) *Determination*. —The term of a patent that is in force on or that results from an application filed before the date that is 6 months after the date of the enactment of the Uruguay Round Agreements Act shall be *the greater* of the 20-year term as provided in subsection (a), or 17 years from grant, subject to any terminal disclaimers. (Emphasis added).

Under this provision, "'[p]atentees need not make any election to be entitled to the longer term.'" *Bayer AG v. Carlsbad Tech., Inc.*, 298 F.3d 1377, 1380 (Fed. Cir. 2002) (quoting 60 Fed. Reg. 20195 at 20207 (April 25, 1995)). The longer term is "'*automatic by operation of law*,'" *id.* (quoting 60 Fed. Reg. 20195 at 20207 (emphasis added by this Court)), and "guaranteed" by statute. *Merck*, 80 F.3d at 1547.

C. Obviousness-Type Double Patenting for Post-URAA Patents: The *Gilead* Decision

For post-URAA patents, obviousness-type double patenting is a "less significant" problem than for pre-URAA patents "'because of the change in the Patent Act from a patent term of seventeen years from issuance to twenty years from filing.'" *Abbvie, Inc. v. Mathilda & Terence Kennedy Inst. of Rheumatology Trust*, 764 F.3d 1366, 1373-74 (Fed. Cir. 2014) (citation omitted). Post-URAA patents that cover similar subject matter and are commonly owned generally expire on the same date, 20 years from the filing date of their earliest priority application, so long as they share the same priority application. However, post-URAA patents still may raise double patenting concerns if the patentee tries to manipulate its patents' terms by having patents on similar subject matter claim priority to different applications with different earliest priority dates, so that a patent claiming priority to a later-filed application would expire later.

Gilead, 753 F.3d 1208, addresses that problem. The post-URAA patents in *Gilead* "issued to the same inventors," were "commonly owned" and "disclose[d] similar content." However, they did "not claim priority to a common patent application." *Id.* at 1209-10. Instead, Gilead "crafted [ ] separate 'chain[s]' of applications," with the '483 patent that Gilead asserted in litigation having "a later priority date than the '375 patent family," and thus expiring later. *Id.* at 1210. This kind of manipulation can only arise with two "post-URAA patents," whose term by statute is "twenty years from the filing date of the earliest application to which the inventor claims priority." *Id.* at 1215. "Because [Gilead's] patents d[id] not claim priority to any common application, they [would] expire at different times as governed by the provisions of [§ 154(a)(2)]," *id.* at 1210, with the '483 patent on which Gilead sued expiring after its '375 patent, as shown below:



This Court rejected Gilead's attempt to extend its term of protection. This Court stated: "[I]t is a bedrock principle of our patent system that when a patent expires, the public is free to use not only the same invention claimed in the expired patent but also obvious or patentably indistinct modifications of that invention." *Gilead*, 753 F.3d at 1214. On the facts in *Gilead*, this Court held that an improper extension of the patent term for a post-URAA patent that expires 20 years from its earliest priority application can be shown by a later expiration date, rather than a later issue date. *Id.* at 1212. At the same time, this Court carefully and repeatedly limited *Gilead*'s holding to the specific "*circumstances of th[at] case*." *Id.* (emphasis added); *see also id.* at 1217. This Court summarized the relevant principle for post-URAA patents in its later decision in *Abbvie*, 764 F.3d at 1373 (bracketed material added)[5]:

> [W]here, as here, the applicant chooses to file separate applications for overlapping subject matter and to claim different priority dates for the applications, the separate patents will have different expiration dates since the patent term [for post-URAA patents] is measured from the claimed priority date. When such situations arise, the doctrine of obviousness-type double patenting ensures that a particular invention (and obvious variations thereof) does not receive an undue patent term extension.

[5] As in *Gilead*, the patentee in *Abbvie* had its post-URAA patents claim different priority dates, with the patent asserted in litigation claiming the later priority date and thus having a later expiration date. *Abbvie*, 764 F.3d at 1373 n.2.

As *Gilead* explains, any other rule for post-URAA patents would invite "significant gamesmanship" by allowing inventors to "orchestrate patent term extensions by (1) filing serial applications on obvious modifications of an invention, (2) claiming priority to different applications in each, and then (3) arranging for the application claiming the latest filing date to issue first." 753 F.3d at 1215.

D. Obviousness-Type Double Patenting is Inapplicable in the Hybrid Situation Presented Here

At the beginning and the end of its analysis, this Court limited its holding in *Gilead* to the "*circumstances of th[at] case*." 753 F.3d 1212; *see also id.* at 1217 (emphasis added). This case, as the district court recognized, is "factually different than *Gilead*," so that *Gilead* does not control the outcome here. Appx675. In *Gilead*, the patent-in-suit was a *post*-URAA patent with a term of 20 years from filing. Here, in contrast, the '471 Patent-in-suit is a *pre*-URAA patent, whose statutory term of 17 years from issuance is "inextricably intertwined with [its] issuance date." *Gilead*, 753 F.3d at 1214-15. As *Gilead* recognized, this distinction is "critical to a double patenting analysis." *Id*. The distinction is all the more important here because Janssen, unlike the patentee in *Gilead*, did not craft separate chains of applications or otherwise do anything to extend its period of exclusivity. The only reason the '471 Patent has a longer term than the '444

Reference Patent is that Congress, in enacting § 154(c)(2), decided to "preserve[ ]" the traditional term of 17 years from issuance for pre-URAA patents if that is "longer than 20 years from filing [of the patent's earliest priority application]." *Merck*, 80 F.3d at 1547.

In extending *Gilead* to the different circumstances of this case, the district court placed undue weight on the "bedrock principle" that "when a patent expires, the public is free to use not only the same invention claimed in the expired patent, but also obvious or patentably indistinct modifications of that invention." Appx675-676 (quoting *Gilead*, 753 F.3d at 1214). But as *Gilead* recognizes, 753 F.3d 1215 n.5, this "bedrock principle" is something of an overstatement. The grant of a patent confers the "right to exclude others" from practicing the claimed invention, 35 U.S.C. § 154(a)(1), but "does not provide [even] the patentee with an affirmative right to practice the patent …." *TransCore, LP v. Elec. Transaction Consultants Corp.*, 563 F.3d 1271, 1275 (Fed. Cir. 2009). *A fortiori*, a patent's expiration does not give anyone the right to practice the invention. "The expiration of a patent right is not a guarantee of a right to use." *Studiengesellschaft Kohle mbH v. N. Petrochem. Co.*, 784 F.2d 351, 357 (Fed. Cir. 1986). Thus, the public is not free to practice an invention if there are "other [dominating] patents still in

force when a patent expires," *id*., or if an act of Congress limits the public's freedom to practice an expired patent.[6]

Gilead itself recognizes situations where "[t]he public's ability to practice an invention claimed in an expired patent may be … restricted," ***e.g.***, by patents covering "separately patented improvements," 753 F.3d 1215 n.5, or by related patents that qualify for term extensions, *id.* at 1215 n.6. *Gilead* actually relied on a narrower, well-established principle: "[T]he point of the double patenting doctrine is to protect the public from attempts by inventors to effectively extend their patent term through a later-expiring patent claiming patentably *indistinct* subject matter." *Id.* at 1215 n.5 (emphasis in original). Likewise, in restating *Gilead*'s holding in *Abbvie*, this Court focused on whether the applicant "receive[d] an *undue* term extension" by "choos[ing] to file separate applications for overlapping subject matter and to claim different priority dates for the applications …." 764 F.3d at 1373 (emphasis added).

This Court's focus in *Gilead* and *Abbvie* on attempts by inventors to unduly extend the patent term returns the analysis to the two principles that have

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[6] For example, under the Biologics Price Competition and Innovation Act of 2009, FDA approval to market a biosimilar "may not be made effective … until 12 years after" the FDA approved the innovator product, giving the innovator "up to twelve years of exclusivity against follow-on products," even if the innovator's patents have all expired. *Amgen, Inc. v. Sandoz, Inc.*, 794 F.3d 1347, 1352 (Fed. Cir. 2015) (quoting 42 U.S.C. § 262(k)(7)(A)).

always guided the double patenting analysis and permit the "consistent application" of the doctrine, before and after the URAA. *Gilead*, 753 F.3d at 1216. These principles are: (1) "'prevent[ing] unjustified time-wise extension of the right to exclude,'" and (2) "prevent[ing] multiple infringement suits by different assignees asserting essentially the same patented invention." *Hubbell*, 709 F.3d at 1145 (citation omitted).

Neither principle is implicated here. The first principle is inapplicable because Janssen did not seek or obtain an "unjustified time-wise extension of the right to exclude." *Id*. The applications for the '471 and '444 Patents both claim the same priority date and there is nothing "unjustified" about the '471 Patent having the longer term that Congress decreed. The second principle is also inapplicable. During prosecution of the '444 Patent, Janssen filed a terminal disclaimer stating that the '444 Patent "shall be enforceable only for and during such period that [the '444] and the ['471] patents are commonly owned." Appx2272-2290 at Appx2289, Appx2292. This eliminated any risk of parties being subjected to multiple suits by different assignees asserting essentially the same invention.

Here, it is an act of Congress that precludes the public from practicing the '471 Patent after the '444 Patent expired. In enacting § 154(c)(1), Congress decided to protect the justified expectations of parties who applied for patents

before the URAA's effective date by allowing those patents to retain the traditional term of 17 years from issuance or to have terms of 20 years from filing, *whichever is greater*. *Merck*, 80 F.3d at 1547. Section 154(c)(1) thus reflects Congress' determination that honoring the expectations of owners of pre-URAA patents was more important than making inventions available to the public at the earliest possible date. That decision by Congress is the reason the '471 patent has a longer term than the '444 patent.

When Congress enacted § 154(c)(1), it was obvious that post-URAA patents in the same family as pre-URAA patents could expire earlier, 20 years from their earliest priority application. Congress could have limited the terms of patents issuing on pre-URAA applications to allow the public to practice those inventions sooner. That would have made the term of pre-URAA patents the same as post-URAA patents, but would have been unfair to innovators who justifiably relied on prior law. Instead, Congress decided that pre-URAA patents can retain the traditional longer term of 17 years from issuance, and thus expire later than related post-URAA patents that share the same priority application.

In these circumstances, the "bedrock principle" that should control the analysis is the core principle of separation of powers. Obviousness-type double patenting is a "judicially-created doctrine." *Eli Lilly*, 251 F.3d at 967. When courts apply that judicially-created doctrine, they "are not at liberty to jettison

Congress' judgment," *Petrella v. Metro-Goldwyn-Mayer, Inc.*, 134 S. Ct. 1962, 1967 (2014), that a pre-URAA patent can have a longer term than a post-URAA patent that shares the same priority application.

The district court's decision frustrates § 154(c)(1)'s purpose and "jettison[s] Congress' judgment" in enacting that provision. Under the district court's reasoning, patentees would have to file terminal disclaimers, disclaiming the portion of the term of pre-URAA patents that extends beyond the expiration of any related post-URAA patent. This would effectively limit pre-URAA patents to the shorter term of post-URAA patents, even though Congress determined, in § 154(c)(1), that the term of pre-URAA patents "shall be the greater" of 17 years from issuance or 20 years from filing.

In addition, the URAA was not intended to "disturb the consistent application of the doctrine of double patenting," *Gilead*, 753 F.3d at 1216, but the district court's decision also has that effect. For pre-URAA patents with a term of 17 years from issuance:

> "[A]ll proper double patenting rejections … rest on the fact that a patent has been issued and later issuance of a second patent will continue [patent] protection, beyond the date of expiration of the first patent" of the same invention or an obvious variation thereof.

Sun, 611 F.3d at 1389 (citation omitted).[7] The district court's decision runs counter to this rule. The issuance of the '444 Patent did not "continue [patent] protection, beyond the expiration of the first patent," *id.*, because the '444 Patent issued after the '471 Patent and expired earlier, so that the term of the '471 Patent is exactly the same as if the '444 Patent never issued. It has not been extended.

This Court and its predecessor courts also have long recognized that obviousness-type double patenting is concerned with the "*improper* extension" of patent rights. *Eli Lilly v. Teva Parenteral*, 689 F.3d at 1379; *see also Eli Lilly v. Barr*, 251 F.3d at 968; *In re Thorington*, 418 F.2d 528, 538 (C.C.P.A. 1969). As the C.C.P.A. stated: "The fundamental reason for the rule is to prevent *unjustified* timewise extension of the right to exclude granted by a patent no matter how the extension is brought about." *Application of Schneller*, 397 F.2d 350, 354 (C.C.P.A. 1968); *see also Van Ornum*, 686 F.2d at 943-44. This Court has agreed, and has equated obviousness-type double patenting with "*improper* extension" of the patent term. *Boehringer*, 592 F.3d at 1346; *Perricone v. Medicis Pharm.*

[7] *See Eli Lilly & Co. v. Teva Parenteral Meds., Inc.*, 689 F.3d 1368, 1376 (Fed. Cir. 2012) ("The doctrine of obviousness-type double patenting is intended to 'prevent the extension of the term of a patent … by prohibiting the issuance of the claims in a *second patent* not patentably distinct from the claims of the *first patent*.'") (quoting *In re Longi*, 759 F.2d 887, 892 (Fed. Cir. 1985)) (emphasis added).

Corp., 432 F.3d 1368, 1375 (Fed. Cir. 2005); *Applied Materials*, 98 F.3d at 1577; *see Kaplan*, 789 F.2d at 1576-77 (describing the phrase "'*improper* extension of monopoly'" as a more apt term than obviousness-type double patenting). "[O]nly if the extension of patent right is *unjustified* is a double patenting rejection appropriate." *In re Braat*, 937 F.2d 589, 595 (Fed. Cir. 1991) (all emphases added).

The longer term of the '471 Patent does not represent an "*unjustified*" or "*improper*" extension of the patent term. It results solely from Congress' decision, in § 154(c)(1), to "preserve[] a guaranteed 17-year [from issuance] term" for patents issuing from applications filed before June 8, 1995 "if [that term] is longer than 20 years from filing …." *Merck*, 80 F.3d at 1547. The district court's ruling that the '471 Patent is invalid for obviousness-type double patenting in view of the '444 Patent, was erroneous and should be reversed.

III. In the Safe Harbor SJ Ruling, the District Court Erred in Granting Summary Judgment of Invalidity for Obviousness-Type Double Patenting Based on the '272 and '195 Patents

The district court also erred in ruling that the asserted claims of the '471 Patent are invalid for obviousness-type double patenting in view of the '195 and '272 Patents.

A. The District Court Erred in Ruling on Summary Judgment

that the § 121 Safe Harbor is Inapplicable[8]

"Prior to the 1952 Patent Act, no protection was afforded to patent applications filed as a result of a restriction requirement—referred to at the time as a 'requirement for division'—and such applications were often rejected or held invalid on double patenting grounds." *Pfizer*, 518 F.3d at 1360-61. "Thus, although a requirement for division embodied a determination by the PTO that the patent application contained more than one patentably distinct invention, such a determination did not protect the divisional application from rejection on grounds of double patenting." *Id.* at 1361. "The inequity of this practice was well known by 1952." *Id*.

Section 121 of the patent statute was intended "to eliminate this inequity and thereby allow applicants to reasonably rely on restriction requirements." *Pfizer*, 518 F.3d at 1361. Section 121's third sentence provides in pertinent part that a patent issuing on an application filed as a result of a restriction requirement "shall not be used as a reference … against a divisional application … if the divisional application is filed before the issuance of the patent on the other application." 35 U.S.C. § 121.

[8] The applicability of the statutory safe harbor is also discussed in Janssen's co-pending appeal, No. 17-1257.

Properly applied, the § 121 safe harbor insulates the '471 Patent from assertions of double patenting based on the '195 and '272 Reference Patents. Janssen filed the application for the '471 Patent before the issuance of the '195 and '272 Reference Patents, as a result of a restriction requirement in the '413 Parent Application, to pursue claims on the non-elected Group I invention of the restriction. The '471 Patent's claims are consonant with Group I of the restriction and fully supported by the original disclosure of the '413 Parent Application. No claims of the '471 Patent are directed at any supplemental disclosures. Moreover, the disclosure of the '471 Patent was amended in reexamination, with the permission of the PTO Director, to conform it to the disclosure of the '413 Parent Application and to designate the '471 Patent a divisional of the '413 Parent Application, so that the '471 Patent is a divisional in form as well as in substance.

In rejecting the applicability of the safe harbor on summary judgment, the district court misread the decisions in *Pfizer*, 518 F.3d 1353, *Amgen*, 580 F.3d 1340, and *G.D. Searle LLC v. Lupin Pharms., Inc.*, 790 F.3d 1349 (Fed. Cir. 2015), as "establish[ing] a bright line rule that only patents resulting from applications *filed as divisional[s]* are protected by the §121 safe harbor." Appx33 (emphasis added). Applying that "bright line rule," the district court concluded that the '471 Patent "is not protected by the §121 safe harbor" because the application for that patent originally was labeled a CIP, rather than a divisional. *Id*.

However, *Pfizer*, *Amgen* and *Searle* do not create the "bright line rule" that the district court applied. Those cases all depended on their facts, which are unlike the facts here. In *Pfizer*, the claims in the patent-in-suit were supported only by new matter added in a CIP application. The only justification this Court identified for excluding the subject CIP from § 121 was that there is "no possible reason for protecting the *new matter* [added in a CIP] from double patenting rejections." *Pfizer*, 518 F.3d at 1362 (emphasis added). This rationale is inapplicable here because the claims of '471 Patent are fully supported by the original disclosure of the '413 Parent Application; Janssen did not seek any claims based on any new disclosures. (Celltrion disputed this, Appx4429, raising a fact issue inappropriate for resolution on summary judgment.).

Searle involved the same patent that previously was invalidated in *Pfizer* after it was reissued as a divisional, deleting the new matter and cancelling claims that depended on it. This Court found that "[f]airness to the public" did not permit application of the safe harbor when, to obtain a reissue of the previously invalidated patent, the patentee retroactively cancelled claims directed to "new matter that was not present" in the parent application, where those claims already had "enjoyed years of patent protection" and had precluded the public "[from] practic[ing] that new matter." *Searle*, 790 F.3d at 1355. This "fairness" concern is not implicated here because no claims in the '471 Patent were directed to or relied

on any new matter. (Again, Celltrion disputed the facts, Appx4429, raising issues that cannot be resolved on summary judgment.).

In the remaining case, *Amgen*, this Court did not simply rely on Amgen's decision to label its applications as continuations rather than divisionals, but instead relied on Amgen's failure to come forward with "any persuasive reason as to why we should deem [those] applications divisional applications for purposes of § 121." *Amgen*, 580 F.3d at 1354.

In contrast, there are powerful reasons why the '471 Patent should be considered a divisional for purposes of § 121. First, Janssen filed the application for the '471 Patent in response to a restriction requirement in the '413 Parent Application, and advised the PTO that it was pursuing the application "pursuant to the restriction requirement set forth in [a] parent application." Appx3938; *see also* Appx3980. Second, the issued claims are fully supported by the original disclosure of the '413 Parent Application and consonant with non-elected Group I of the restriction in the Parent Application. Third, the application was prosecuted as a divisional for purposes of its claims. Appx3789-3790. Fourth, because the newly added disclosure was not the subject of any claims its disclosure only benefited the public, by increasing available knowledge. *See Johnson & Johnston Assocs. v. R.E. Serv. Co.*, 285 F.3d 1046, 1054 (Fed. Cir. 2002) (en banc) ("[W]hen a patent drafter discloses but declines to claim subject matter … this action

dedicates that unclaimed subject matter to the public."). Fifth, the '471 disclosure was amended during reexamination, with approval of the PTO Director, to delete the supplemental disclosure, to conform it in substance with that of the '413 Parent Application and designate the application a divisional of the '413 Parent Application. Appx4104-4105. On these facts, the '471 Patent is a divisional both in form and in substance. The statutory safe harbor is properly applicable here.

At a minimum, the district court erred in brushing aside disputed fact issues about the safe harbor's applicability, including issues concerning: (1) PTO and practitioner practice in the 1990s in treating continuations, CIPs and divisionals as interchangeable for purposes of § 121, Appx3789-3790; (2) Janssen's reliance on that practice and the reasonableness of that reliance, Appx4110-4111; Appx3788; (3) Janssen's reliance on the Examiner's withdrawal of a double-patenting rejection after Janssen asserted its entitlement to the safe harbor, Appx4113; (4) the absence of claims in the '471 patent that are supported by additional matter not present in the '413 Parent Application; and (5) the expert opinion of former Deputy Commissioner Kunin that "[i]n effect, the '471 Patent was a divisional of the ... Parent Application as far as its claims are concerned," Appx3789-3790. Viewing these disputed facts "in the light most favorable to [Janssen]," *MRC*, 747 F.3d at 1331, the district court erred in holding on summary judgment that the safe harbor is inapplicable.

B. The District Court Erred in Holding that the One-Way Test Is Applicable

Even if the safe harbor were not available, it was error to grant summary judgment that the '471 Patent is invalid for obviousness-type double patenting in view of the '195 and '272 Reference Patents.

"This court has set forth two tests for obviousness-type double patenting": the "one-way test" and the '"two-way patentability test." *In re Emert*, 124 F.3d 1458, 1461 (Fed. Cir. 1997). Where the one-way test applies, the issue is whether a challenged claim would have been obvious over the claims of a reference patent. *In re Berg*, 140 F.3d 1428, 1432 (Fed. Cir. 1998). In contrast, "[u]nder the two-way analysis, this court examine[s] each claim to determine whether it was an obvious variant of the other …." *Emert*, 124 F.3d at 1461. Thus, under the two-way test, a challenged claim would only be invalid if: (1) the claim in question would have been obvious over the claims of a reference patent; and (2) vice versa. *Berg*, 140 F.3d at 1432. As a result, claims that would be invalid under the one-way test may be valid under the two-way test. *Id*.

The two-way test applies where two applications issue in an order that is prejudicial to the scope of protection for the later-issued patent and "the PTO is solely responsible for the delay in causing the second-filed application to issue prior to the first." *Hubbell*, 709 F.3d at 1149 (quoting *Berg*, 140 F.3d at 1437).

"Th[e] two-way analysis is necessary because a later-filed improvement patent may issue before an earlier filed basic invention." *In re Goodman*, 11 F.3d 1046, 1053 (Fed. Cir. 1993). "The rationale [for the two-way test] is that an applicant … who files applications for basic and improvement patents should not be penalized by the rate of progress of the applications though the PTO, a matter over which the applicant does not have complete control." *Braat*, 937 F.2d at 593. This rationale is fully applicable where the applications are filed on the same day and an obviousness-type double patenting challenge is directed at a basic patent that issued later due solely to delays caused by the PTO in examination during "the critical co-pendent period" when both applications were co-pending in the PTO. *Basell Poliolefine*, 547 F.3d at 1376.

Here, the district court found that "there may be a genuine dispute of fact concerning whether the PTO is solely responsible for the '471 Patent issuing after the '195 and '272 Patents." Appx5. On Celltrion's summary judgment motion, this determination should have mandated application of the two-way test. However, the district court held that the two-way test is inapplicable because "the application for the '471 Patent was filed on the same day as the application for the '272 Patent." *Id*. Based on that same-day filing, the district court concluded that "[t]he PTO did not decide the applications in the reverse order of filing" and "[t]herefore, the two-way test is not applicable." *Id.*

Although the applications for the '471 and '272 Patents were filed on the same day, the two way test is not intended to reward or punish order of filing. Rather, it is intended to prevent a later-issued patent from being invalid for obviousness-type double patenting where its later issuance occurs through no fault of the applicant, and the later-issued patent would otherwise have been entitled to the full scope of its claims had the order of issuance been reversed. *Goodman*, 11 F.3d at 1053; *Braat*, 937 F.2d at 593.

Same-day filing is fully compatible with this rationale. In a perfect world, applications filed on the same day would be examined at the same rate and issue on the same day. But when they do not, solely because of PTO-determined examination rates, the two-way test should be available as protection against assertions of double patenting. Any other result would unfairly deny protection to applicants whose patents were delayed in issuing through no fault of the applicants.

The fact that the applications leading to the '471 and '272 Patents could not have been filed as a single application (due to the restriction in the '413 Parent Application) and were filed on the same day should strengthen, not weaken, their eligibility for the two-way test. Neither application qualifies as prior art against the other, and it would be unfair to limit the protection accorded the later-issuing patent where, as here, (a) the applications could not have been filed as a single application due to a prior restriction requirement preventing them from

being combined; (b) its later issuance resulted solely from the PTO's conduct in examining the applications; and (c) both patents would have been entitled to the full scope of protection claimed if the order of issuance were reversed because as here, the claims of the earlier-issued patent are not obvious in view of the claims of the later-issued patent.

Even if this Court were to conclude that filing order is dispositive, it should treat the application for the '471 Patent as having been filed before the application for the '272 Patent, as reflected by the fact that it has a lower serial number than the application for the '272 Patent (No. 08/192/093, compared to No. 08/192,102). In addressing the applicability of the two-way test, this Court has never required some set period of time—be it a day or a week or a month—between the filing of the two applications. If the two-way test is deemed to require that the later-issuing application be filed before the earlier-issuing application, then earlier filing by serial number should suffice. In effect, the district court decided that "before" means at least one day before, and not simply "before." There are circumstances in which a statute or rule requires an event to take place at least one day before another date or event.[9] But the two-way test is judge-made law and imposes no such requirement.

[9] *See*, *e.g.*, *United States v. Locke*, 471 U.S. 84, 96 (1985) (addressing the phrase "prior to December 31" in 15 U.S.C. § 78n(e)), *Burton v. Stevedoring Servs. of*

This Court recently rejected such an argument in *Immersion Corp. v. HTC Corp.*, 826 F.3d 1357 (Fed. Cir. 2016). The question there was entitlement to an earlier application's filing date under 35 U.S.C. § 120, which refers to an application filed "before the patenting" of the prior application. The accused infringer argued that when two events (issuance of a patent and filing of a continuation from the application for that patent) both occur on the same day then neither event occurs "before" the other. This Court disagreed. It held that a continuation application is "filed before the patenting [of another application]" where both events occur "on the same day," so long as the continuation is filed before the time the patent issues. 826 F.3d at 1362-63.

The same reasoning should apply here. Since the processing order of same-day filed applications is entirely with the PTO's control, applicants should be given the benefit of treating either application as having been filed before the other if doing so will preserve a patent's validity.

By the same token, in addressing the applicability of the two-way test for double patenting, there is no rule or policy that requires filing on separate days. It is sufficient that one application be filed before another application, even if both are filed on the same day. If so, and if the applications are ultimately decided in

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Am., 196 F.3d 1070, 1073 (9th Cir. 1999) (addressing the phrase "before September 12, 1996" in the Department of Labor Appropriations Act of 1996).

the "reverse order of filing," *Hubbell*, 709 F.3d at 1149, then the two-way test applies.

The district court cited MPEP § 804.II(B)(2)(b), which states that "[i]f both applications are filed on the same day, only a one-way determination of distinctness is needed in resolving the issue of double patenting." Appx35 (quoting MPEP § 804.II(B)(2)(b)). That provision is "not binding on this court," *Hubbell*, 709 F.3d at 1146, and it incorrectly applies the law. The only authority the MPEP cites for this proposition is *Berg*, 140 F.3d 1428, but as the district court noted, "*Berg* does not control because the issue, although presented, was not decided." Appx35. As the district court explained, *Berg* holds that "'simultaneously filing two separate applications *that could have been filed as one application* disqualifies the applicant from the two-way test,'" Appx36 (quoting *Berg*, 140 F.3d at 1435) (emphasis added). *Berg*'s holding has no application here because the restriction requirement prevented Janssen from filing all of its claims in "a single application." Appx36-37. As the district court correctly noted, *Berg* "neither accepted nor rejected" the theory that the two-way test is inapplicable "[where] the applications at issue were filed on the same day." *Id*.

Here, the application for the '471 Patent was filed by serial number before the application for the '272 Patent, albeit on the same day, and it was filed more than eight months before the application for the '195 Patent. The PTO then

decided the applications for the '272 and '195 Patents in the reverse order of filing with respect to the application for the '471 Patent, with the '272 and '195 Patents issuing in 1997 and the '471 Patent issuing in 2001.

On these facts, the two-way test applies if "'the PTO is solely responsible for the delay in causing the second-filed application[s] [for the '195 and '272 Patents] to issue prior to the ['471 Patent].'" *Hubbell*, 709 F.3d at 1149 (quoting *Berg*, 140 F.3d at 1437). On summary judgment, the district court's recognition of disputed fact questions on that issue (Appx5) should have precluded application of the one-way test. The district court erred in holding on summary judgment that the one-way test applies here.[10]

C. When the Two-Way Test is Correctly Applied, the Evidence Does Not Support Summary Judgment of Invalidity

The district court also erred in stating that summary judgment of invalidity would be appropriate if the two-way test were applied. In reaching that conclusion, the district court incorrectly compared the claims of the '195 and '272 Patents to the '471 *specification*, when the correct analysis would have compared

[10] In its co-pending appeal, No. 17-1257, Janssen has demonstrated that the PTAB erred in concluding that the PTO was not solely responsible for the delay that caused the applications to issue in the reverse order of filing. The PTAB's determination on this issue has no possible application here because Celltrion, in moving for summary judgment, needed to—but did not—establish its position on this issue by undisputed evidence.

the claims of the '195 and '272 Patents to the '471 *claims*. Under the correct analysis, there is no basis for concluding that the asserted claims of the '471 Patent are invalid under the two-way test.

To satisfy the two-way test, Celltrion needed to demonstrate, on undisputed facts and by clear and convincing evidence: (a) that the asserted claims of the '471 Patent would have been obvious in view of reference claim 6 of the '195 Patent and reference claim 7 of the '272 Patent, and (b) that the converse is true, *i.e.*, that the reference claims of the '195 and '272 Patents would have been obvious in view of the asserted claims of the '471 Patent. *Berg*, 140 F.3d at 1432; *Braat*, 937 F.2d at 593.

Celltrion did not present evidence from any fact witness or expert on obviousness under the two-way test. The only evidence on that issue came from Janssen's experts, Dr. van Deventer and Dr. Ghrayeb. As explained in their declarations, the asserted claims of the '471 Patent do not render the reference claims in the '195 and '272 Patents obvious. *See* Appx3618-3651 at Appx3650-3651; Appx3653-3667 at Appx3665-3666. At a minimum, their declarations raise disputed fact issues that cannot be resolved on summary judgment.

1. The District Court Erred in Comparing the Claims of the '195 and '272 Patents to the '471 Patent's

Specification, Rather than Its Claims

The general rule is that "[d]ouble-patenting is altogether a matter of what is claimed" and "the disclosure of a patent … [*i.e.*, the specification] cannot be used as though it were prior art." *Gen. Foods Corp. v. Studiengesellschaft Kohle mbH*, 972 F.2d 1272, 1281 (Fed. Cir. 1992); *see also Abbvie*, 764 F.3d at 1380 ("[A] reference patent's specification cannot be used as prior art in an obviousness-type double patenting analysis.").

Instead of applying this general rule, the district court invoked an exception that only has been applied in cases—unlike this one—where the claims of a reference patent do not recite *any utility* for a compound described in the claims. *See Sun*, 611 F.3d at 1383; *Pfizer*, 518 F.3d at 1358; *Geneva Pharms., Inc. v. GlaxoSmithKline PLC*, 349 F.3d 1373, 1385-86 (Fed. Cir. 2003). In all of these cases, it was necessary to look to the specification to find some utility for a claimed compound because no utility was disclosed in the claims.

For example, in *Sun*, the claims of the reference patent were directed "solely to [the compound] gembitabine," with no disclosure of any utility in the claims. This Court looked to specification because the reference patent "claimed a compound, disclosing its utility [only] in the specification …." *Id.*, 611 F.3d at 1383-85. Similarly, in *Geneva*, the reference claim described a "compound having

a certain physical property," with no description of any utility in the claims. *Id.*, 349 F.3d at 1385-86. *Pfizer*, 518 F.3d at 1358, presented the same situation.

Here, in contrast, the claims of the '471 Patent expressly recite a utility for the claimed antibodies, *i.e.*, the "capab[ility] of binding an epitope specific to human tumor necrosis factor TNFα …." Appx197. There was no need to consider the '471 specification to find a utility for the claimed antibodies because a utility is recited in the claims. This Court has never applied a specification-to-claims comparison in these circumstances.

The district court was incorrect in stating that "essentially th[is] same argument" was "rejected" in *Sun*. Appx40-41. In *Sun*, and in *Geneva* and *Pfizer*, the reference claims did not recite *any* utility for a claimed compound. None of those cases considered—much less "rejected"—the applicability of the general rule against using a reference patent's specification as prior art where a utility is expressly recited in the reference claims.

On the facts of this case, the applicable rule is the general rule that a patent's *specification* "cannot be used as prior art in an obviousness-type double patenting analysis." *Abbvie*, 764 F.3d at 1380. The exception the district court relied upon is not applicable here.

2. The Antibody Claims of the '471 Patent Do Not Render it Obvious to Use the cA2 Antibody to Treat

Crohn's Disease or Rheumatoid Arthritis

Applying the general rule, the record here cannot support a finding that the *claims* of the '471 Patent—as opposed to its *specification*—render the claims of the '272 and '195 Patents obvious. The asserted claims of the '471 Patent recite certain chimeric antibodies "capable of binding an epitope specific to human tumor necrosis factor TNFα." Appx197. The claims do not describe using those antibodies as part of a method for treating any particular disease.

Janssen's experts, Dr. van Deventer and Dr. Ghrayeb, explained that the claims of the '471 patent would not have made it obvious in 1991 to use the cA2 antibody to treat rheumatoid arthritis (as recited in reference claim 6 of the '195 Patent) or Crohn's disease (as recited in reference claim 7 of the '272 Patent). Appx3618-3651 at Appx3650-3651; Appx3653-3667 at Appx3665-3666. Celltrion did not present any evidence from any fact witness or expert on this issue.

3. Celltrion's Failure of Proof on Obviousness Under the Two-Way Test Requires Reversal

The only evidence on obviousness under the two-way test came from Janssen's experts. Celltrion did not offer any evidence that the claims of the '471 Patent would have rendered the claims of the '195 and '272 Patents obvious.

Expert testimony may not be needed in cases involving simple mechanical technology, *Wyers v. Master Lock Co.*, 616 F.3d 1231, 1240 (Fed. Cir.

2010), but where, as here, the technology is complex and "beyond the comprehension of laypersons," expert testimony can be "essential." *Id.* at 1240 n.5; *see Alexsam, Inc. v. IDT Corp.*, 715 F.3d 1336, 1347-48 (Fed. Cir. 2013) (expert testimony necessary in case involving complex technology); *Kinetic Concepts, Inc. v. Smith & Nephew, Inc.*, 688 F.3d 1342, 1369 (Fed. Cir. 2012) (same).

Celltrion's failure to provide any expert testimony on whether the '272 and '195 claims would have been obvious in view of the '471 claims is fatal to its obviousness theory under the two-way test, and is a further reason why the district court decision should be reversed.

CONCLUSION

This Court should reverse the grant of summary judgment that claims 1, 3 and 5-7 of the '471 Patent are invalid for obviousness-type double patenting.

Dated: January 26, 2017

Respectfully submitted,

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ADDENDUM

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UNITED STATES DISTRICT COURT
DISTRICT OF MASSACHUSETTS

JANSSEN BIOTECH, INC. ET AL.,
Plaintiffs
v. CIVIL ACTION NO. 15-cv-10698-MLW
CELLTRION HEALTHCARE CO., INC. ET AL.,
Defendants

JUDGMENT IN A CIVIL CASE

WOLF, D.J.

Jury Verdict. This action came before the court for a trial by jury. The issues have been tried and the jury has rendered its verdict.

X **Decision by the Court**. This action came to trial or hearing before the Court. The issues have been tried or heard and a decision has been rendered.

IT IS ORDERED AND ADJUDGED

In accordance with the Memorandum & Order dated 9/26/2016, partial final judgment enters in favor of the defendants as to Count 3, that U.S. Patent No. 6,284,471 is invalid.

ROBERT FARRELL,
CLERK OF COURT
By

Dated: September 27, 2016 /s/ Daniel C. Hohler
Daniel C. Hohler, Courtroom Deputy Clerk

UNITED STATES DISTRICT COURT
DISTRICT OF MASSACHUSETTS

JANSSEN BIOTECH, INC. ET AL, Plaintiffs, v. CELLTRION HEALTHCARE CO. INC., ET AL., Defendants.	)	C.A. No. 15-10698-MLW 16-11117-MLW

MEMORANDUM AND ORDER

WOLF, D.J. August 19, 2016

For the reasons described in detail in court on August 17 and August 18, 2016, and summarized below, it is hereby ORDERED that:

1. Defendants' Motion for Summary Judgment of Invalidity of U.S. Patent No. 6,284,471 for Obviousness-Type Double Patenting (the "Gilead Motion") (Docket No. 127) is ALLOWED. Plaintiffs hold U.S. Patent No. 6,284,471 (the "'471 Patent"). The '471 Patent was issued on September 4, 2001. Standing alone, it would expire on September 4, 2018. Plaintiffs previously held U.S. Patent No. 6,790,444 (the "'444 Patent"). The '444 Patent was issued on September 14, 2004 and expired on July 11, 2011. The parties agree that the '471 Patent is not patentably distinct from the '444 Patent. The Court of Appeals for the Federal Circuit held in Gilead Sciences., Inc. v. Natco Pharma Ltd., 753 F.3d 1208 (Fed. Cir. 2014), cert. denied, 135 S. Ct. 1530 (2015), that a later-issuing, earlier-expiring patent can act as a double-patenting reference for an earlier-issuing, later-expiring patent.

The court finds that the reasoning in Gilead applies where, as here, the later-issued patent expires earlier because of the change to patent terms resulting from the Uruguay Round Agreements Act, codified at 35 U.S.C. §154. In essence, the court concludes that the statute was not intended to alter the judicial doctrine of obviousness double-patenting. See Gilead, 753 F.3d at 1216. Therefore, claims 1, 3, 5, 6, and 7 of the '471 Patent are invalid for obviousness-type double-patenting in light of the patentably indistinct, earlier-expiring '444 Patent.

2. Defendants' Motion for Summary Judgment of Invalidity of U.S. Patent No. 6,284,471 for Obviousness-Type Double Patenting Based On U.S Patent No. 5,698,195 and U.S. Patent No. 5,656,272 (the "Reexam Motion") (Docket No. 176) is ALLOWED.

(a) The following facts are undisputed. Claims 1, 3, 5, 6, and 7 of the '471 Patent (the "Asserted Claims") claim a genus of antibodies that encompasses the infliximab, or cA2, antibody. Claim 6 of U.S Patent No. 5,698,195 (the "'195 Patent") recites "[a] method of treating rheumatoid arthritis in a human comprising administering to the human an effective TNF-inhibiting amount of chimeric anti-TNF anti[b]ody cA2." Claim 7 of U.S. Patent No. 5,656,272 (the "'272 Patent") recites "[a] method of treating TNFα-mediated Crohn's disease in a human comprising administering to the human an effective TNF-inhibiting amount of chimeric anti-TNF antibody cA2." The application for the '471

Patent, U.S. Pat. App. No. 08/192,093 (the "'093 Application"), was filed on February 4, 1994, and, as indicated earlier, standing alone would expire on September 4, 2018. The application for '272 Patent was also filed on February 4, 1994. The '272 Patent was issued on August 12, 1997 and expired on August 12, 2014. The application for the '195 Patent was filed on October 18, 1994. The '195 Patent was issued on December 16, 1997 and expired on December 16, 2014.

(b) The '471 Patent is not entitled to the protection of the 35 U.S.C. §121 statutory safe harbor (the "Section 121 safe harbor"). The Section 121 safe harbor applies only to applications filed as "divisional." See Pfizer, Inc. v. Teva Pharm. USA, Inc., 518 F.3d 1353, 1362 (Fed. Cir. 2008). The '093 Application was filed as a continuation-in-part of U.S. Pat. App. No. 08/013,413 (the "'413 Application"). It was not filed as divisional of the '413 Application. The court does not have discretion to deem the '093 Application divisional. See id.; Amgen Inc. v. F. Hoffman-La Roche Ltd, 580 F.3d 1340, 1354 (Fed. Cir. 2009)

(c) The one-way test for obviousness applies to this motion. Under that test, the Asserted Claims are obvious in light of the '195 and '272 Patents. "The two-way test . . . is 'a narrow exception to the general rule of the one-way test . . .' In re Hubbell, 709 F.3d 1140, 1149 (Fed. Cir. 2013) (quoting In re Berg, 140 F.3d 1428, 1432 (Fed. Cir. 1998)). It "is appropriate only in

the 'unusual circumstance' where 'the PTO is solely responsible for the delay in causing the second-filed application to issue prior to the first.'" Id. (quoting Berg, 140 F.3d at 1437). However, the application for the '471 Patent was filed on the same day as the application for the '272 Patent. The PTO did not decide the applications in the reverse order of filing. Therefore, the two-way test is not applicable. See United States Patent and Trademark Office, Manual of Patent Examining Procedure (9th ed. Nov. 2015) §804.II(B)(2)(b). Plaintiffs acknowledge that under the one-way test, the Asserted Claims are invalid for obviousness-type double-patenting in light of the '195 Patent and the '272 Patent.

(d) Viewing the evidence in the light most favorable to plaintiffs, there may be a genuine dispute of fact concerning whether the PTO is solely responsible for the '471 Patent issuing after the '195 and '272 Patents. However, any such dispute would not be material because assuming, without finding, that the two-way test applied, the Asserted Claims would also be obvious in light of the '195 Patent and the '272 Patent under that test. Under the two-way test, the court performs the one-way analysis and also analyses "whether the [reference] patent claims are obvious over the [challenged patent] claims." Hubbell, 709 F.3d at 1149 (quotations omitted). Under Sun Pharm. Indus., Ltd. v. Eli Lilly & Co., 611 F.3d 1381, 1387 (Fed. Cir. 2010), it is proper

for the court to consider any utility disclosed in the '471 Patent specification when analyzing whether the '195 and '272 Patent claims are obvious in light of the '471 Patent. The '471 Patent specification describes the infliximab antibody and discloses the same uses for infliximab that are claimed in the '195 and '272 patents: treatment of Crohn's disease and rheumatoid arthritis. "[A] claim to a method of using a composition is not patentably distinct from an earlier claim to the identical composition in a patent disclosing the identical use." Geneva Pharm., Inc. v. GlaxoSmithKline PLC, 349 F.3d 1373, 1385-86 (Fed. Cir. 2003). The '195 and '272 Patent claims would, therefore, be obvious in light of the '471 Patent. Both prongs of the two-way obviousness test are met. Accordingly, the Asserted Claims are invalid for obviousness-type double-patenting in light of the '195 and '272 Patents under that test as well.

3. The Motion to Supplement Claim Construction Record (Docket No. 217) is ALLOWED.

4. The court construes the disputed term "cell culture media" in claim 1 of U.S. Patent No. 7,598,083 (the "'083 Patent") to mean "nutritive media for culturing cells." This construction is consistent with the plain and ordinary meaning of the term as understood by those skilled in the art. The patentees did alter the meaning of the term by acting as their own lexicographer or disavowing the otherwise broad scope of the term. See Phillips v.

AVH Corp., 415 F.3d 1303, 1316 (Fed. Cir. 2005); Thorner v. Sony Computer Entm't Am. LLC, 669 F.3d 1362, 1365-66 (Fed. Cir. 2012).

5. Plaintiffs' Motion Expedited [sic] Trial On 083 Patent And To Set A Discovery Schedule, If Necessary, For Remaining 471 Patent Issues (Docket No. 166-1) is, as requested by plaintiffs at the August 18, 2016 hearing, WITHDRAWN.

6. Defendants' Cross Motion To Limit Plaintiffs' Remedy As To The '471 Patent (Docket No. 190) is MOOT.

7. Pursuant to the Stipulation of the parties, plaintiffs' Motion For Consolidation And For Bifurcation Of Damages Issues Pursuant To Fed. R. Civ. P. 42 And Local Rule 40.1 (Docket No. 186) is ALLOWED.

(a) Janssen Biotech, Inc. v. Celltrion Healthcare Co., Ltd., Civil Action No. 1:15-cv-10698-MLW and Janssen Biotech, Inc. v. Celltrion Healthcare Co., Ltd., Civil Action No. 1:16-cv-11117-MLW are consolidated for pre-trial and trial proceedings.

(b) All discovery deadlines concerning U.S. Pat. No. 7,598,083 set by the Court in Civil Action No. 1:15-cv-10698 apply equally to both actions.

(c) All issues of damages shall be bifurcated and addressed, if necessary, after liability is decided.

8. Defendants shall, by August 22, 2016, file their motion for judgment pursuant to Fed. R. Civ. P. 54(b).

9. Plaintiffs shall, by August 29, 2016, respond to defendants' motion for entry of judgment.

10. The parties shall, by September 21, 2016, confer and report concerning whether these cases have been settled.

11. If necessary, a pretrial conference shall be held on October 6, 2016, at 2:30 p.m. The parties shall, by September 27, 2016, confer and file, jointly if possible, separately if necessary, a proposed agenda for the conference and also file pretrial memoranda addressing, to the extent possible, items (1) through (10) of the attached Procedural Order.

12. Trial shall commence on February 13, 2017, at 9:30 a.m.

UNITED STATES DISTRICT JUDGE

UNITED STATES DISTRICT COURT
DISTRICT OF MASSACHUSETTS

Janssen Biotech, Inc. et al.
Plaintiff

V.

Celltrion Healthcare Co. Inc., et al.
Defendant

CIVIL ACTION

NOs. 15-10698; 16-11117

PROCEDURAL ORDER
RE: FINAL PRETRIAL CONFERENCE/TRIAL

Wolf, D. J.

The above-entitled action is scheduled for a final pre-trial conference on_____ at _____ in Courtroom #10 on the 5th Floor. Counsel shall be prepared to commence trial of this action on or after _____. Each party shall be represented at the pretrial conference by trial counsel.

In order to secure the just, speedy and inexpensive determination of this action in accordance with the Civil Justice Reform Act of 1990 and Local Rule 16.5, the parties shall meet prior to this conference to accomplish the following:

(1) to discuss and negotiate settlement of the action;
(2) to draft and sign a stipulation as to all uncontested facts;
(3) to narrow the issues to be tried;
(4) to exhibit to all parties any and all photographs, documents, instruments and other objects any party intends to offer as exhibits at trial;
(5) to give notice to all parties of the names and addresses of witnesses a party intends to call at trial, including the names and qualifications of any expert witnesses.

Counsel shall prepare and file, either jointly or separately, pretrial memoranda and/or trial documents which set forth the following:

(1) a concise summary of the evidence that will be offered by the plaintiff, defendant and other parties with respect to both liability and damages (including special damages, if any);
(2) a statement of facts established by the pleadings, by admissions or by stipulations. Counsel shall stipulate all facts not in genuine dispute;
(3) contested issues of fact;
(4) any jurisdictional questions;
(5) any question raised by pending motions;
(6) issues of law, including evidentiary questions, together with supporting authority;
(7) any requested amendments to the pleadings;
(8) any additional matters to aid in the disposition of the action;
(9) the probable length of trial and whether jury or nonjury;
(10) a list of the names and addresses of witnesses who will testify at trial and the purpose of the testimony, i.e., whether factual, medical, expert, etc.;
(11) a list of the proposed exhibits (photographs, documents, instruments, and all other objects) in order of their introduction to the Court. Those exhibits to be introduced without objection shall be identified by a single sequence of numbers and those items to which a party reserves the right to object shall be identified by a single sequence of capital letters, regardless of which party is offering the exhibit.

This material shall be filed, in duplicate, no later than five (5) business days prior to the scheduled date for the initial pretrial conference. A party who intends to object to any proposed exhibit or witness shall give written notice to all parties setting forth the basis for the objection and file said

notice, in duplicate, with the clerk on or before_____ A party who intends to file any motion in limine shall do so no later than _____. Any responses to a motion in limine shall be filed no later than_____.

Five (5) business days prior to the date assigned for trial each party shall file in duplicate:

(A) In cases to be tried to a jury, a trial brief including:

(1) any proposed questions for the voir dire examination of the jury;
(2) requests for instructions to the jury with citation to supporting authority;
(3) any proposed interrogatories or special verdict form.

(B) In nonjury cases, a trial brief including:

(1) any proposed findings of fact and requested rulings of law.

If the trial materials required by this Order have been previously filed with the Court, please advise the Court in writing of the filing date and supplement trial documents, as necessary. Immediately upon receipt of this Order, any counsel who realizes that one or more attorneys have not been notified shall forthwith notify the additional attorney(s) in writing as to the entry of this Order and file a copy of the writing with the clerk.

Compliance with this Order is not excused, absent the actual filing of closing papers or the entry of a Settlement Order of Dismissal in a form prescribed by the Court.

PLEASE NOTE: The Court requires twenty-four hour notice of settlement. Any settlement on the eve of trial may result in the imposition of costs, including the costs associated with bringing in jurors unnecessarily.

By the Court,

_____ _____

Date Deputy Clerk

Copies To:

(Pretrial.ord - 09/92) [proco.]

UNITED STATES DISTRICT COURT
DISTRICT OF MASSACHUSETTS

JANSSEN BIOTECH, INC. ET AL, Plaintiffs, v. CELLTRION HEALTHCARE CO. LTD., ET AL., Defendants.	))))))))	C.A. No. 15-10698-MLW

MEMORANDUM AND ORDER

WOLF, D.J. September 28, 2016

This Memorandum is based on the transcript of the decision rendered orally on August 17, 2016, allowing defendants Celltrion Healthcare Co., Ltd., Celltrion, Inc., and Hospira, Inc.'s (collectively "Celltrion") Motion for Summary Judgment of Invalidity of U.S. Patent No. 6,284,471 for Obviousness-Type Double Patenting (the "Gilead Motion"). This Memorandum adds citations, and clarifies and amplifies some language.

* * * *

I. SUMMARY

Plaintiffs Janssen Biotech, Inc. and New York University, (collectively "Janssen"), are the holders of patents related to a biologic medication called Remicade, which is based on an antibody called infliximab. Plaintiffs allege that Celltrion has infringed these patents by filing an abbreviated Biologic License Application for a product that is "biosimilar" to Remicade.

Two patents are at issue in this motion for summary judgment based on obviousness-type double patenting. They are U.S. Patent Number 6,284,471 (the "'471 patent" or "'471"), and U.S. Patent Number 6,790,444 (the "'444 patent" or "'444"). The '471 patent covers a genus, or group, of compounds that includes infliximab. The '444 patent is for the infliximab antibody specifically.

Plaintiffs concede that the '444 claims are not patentably distinct from the '471 claims. Both patents are based on an application filed in 1991, which is sometimes called the "priority application." The priority date for each patent is 1991.

The '471 patent was filed in 1994, and issued on September 4, 2001. If it stood alone, it would expire on September 4, 2018 because it was filed before the 1995 effective date of the law altering patent terms, the Uruguay Round Agreements Act (the "URAA"). The URAA is codified at 35 U.S.C. §154, most pertinently at §154(c)(1). The URAA provides protection for 20 years from the date of the original, priority application or 17 years after issuance, whichever is longer, for applications filed before 1995. Therefore, if the '471 patent stood alone, it would expire in 2018. However, for applications filed after 1995, patent protection extends for 20 years after the date the original, priority application was filed. The application for the '444 patent was filed in 2001, after the 1995 effective date of the URAA, and was issued

in 2004. As it was based on a 1991 priority application, it expired 20 years later, in 2011.

In Celltrion's Gilead motion, the defendants seek summary judgment of invalidity on Claims 1, 3, 5, 6 and 7 of the '471 patent for obviousness-type double patenting based on the '444 patent. The only question presented by the motion is whether, in view of the Federal Circuit's decision in Gilead Sciences, Inc. v. Natco Pharma Limited, 753 F.3d 1208 (Fed. Cir. 2014), the earlier-expiring '444 patent should be held to be a double patenting reference that invalidates the '471 Patent. I find that it is such a reference and, therefore, the '471 patent is invalid.

Gilead involved two patents based on applications filed after 1995. Therefore, it did not implicate the provision of the URAA that provides patent protection for at least 17 years after issuance if the application for a patent in dispute was filed before 1995. As this case is factually different than Gilead, Gilead is not binding precedent. I find, however, that in enacting the URAA, Congress and the President did not intend to alter the judicially-created doctrine of obviousness-type double patenting or restrict the power of the courts to apply it to patents resulting from applications filed before 1995.

I also find that the Federal Circuit would apply the Gilead ruling to the circumstances of this case and again find that a later-issued but earlier-expiring patent can serve as a reference

that renders an earlier-issued but later-expiring patent invalid for obviousness-type double patenting.

II. PROCEDURAL HISTORY

On August 16, 2016, the court heard oral argument on the Gilead Motion. On August 17, 2016, the court issued an oral decision, allowing the Gilead Motion. On August 19, 2016, the court issued an order summarizing the reasons for its oral decision. This Memorandum, like the August 17, 2016 transcript, more fully explains those reasons.

III. DISCUSSION

In Gilead, 753 F.3d at 1214, the Federal Circuit wrote that "[i]t is a bedrock principle of our patent system that when a patent expires, the public is free to use not only the same invention claimed in the expired patent but also obvious or patentably indistinct modifications of that invention." The court explained that "[t]he public should . . . be able to act on the assumption that upon the expiration of [a] patent it will be free to use not only the invention claimed in the patent but also [any] modifications or variants [of it] which would have been obvious to those of ordinary skill in the art at the time the invention was made." Id. (citing In re Longi, 759 F.2d 887, 892 (Fed. Cir. 1985)). "The double patenting doctrine has always been implemented to effectively uphold that principle." Id.

Plaintiffs acknowledge that the invention claimed in the '471 patent is an obvious or patentably indistinct modification of the invention claimed in the '444 patent.

In Gilead, the Federal Circuit stated that "the obviousness-type double patenting doctrine prohibits an inventor from extending his right to exclude through claims in a later-expiring patent that are not patentably distinct from the claims of the inventor's earlier-expiring patent." Id. at 1210. The court noted that federal courts had applied this principle for over a century. See id. at 1212. The Federal Circuit essentially rejected plaintiffs' argument here that the URAA manifests a statutory intent to provide patents emerging from applications filed before 1995 with at least 17 years' protection despite the otherwise applicable judicial doctrine of obviousness-type double patenting. See id. at 1216.

The URAA is silent on this issue. It does not state that pre-URAA patents will always have 17 years' protection. Nor does it reference the doctrine of obviousness-type double patenting.

Generally, the Supreme Court "presumes that legislatures act with case law in mind." Abuelhawa v. United States, 129 S.Ct. 2102, 2106 (2009); see also Miles v. Apex, 111 S.Ct. 317, 325 (1990). Consistent with this well-established canon, the Federal Circuit wrote in Gilead that "Congress could not have intended to inject the potential to disturb the consistent application of the doctrine of double patenting by passing the URAA." Gilead, 253

F.3d at 1216. "[T]he primary ill avoided by enforcement of the double patenting doctrine is a restriction on the public's freedom to use the invention claimed in a patent and all obvious modifications after that patent expired." Id. at 1215. Therefore, the Federal Circuit held that "an earlier-expiring patent can qualify as an obviousness-type double patenting reference for a later-expiring patent under the circumstances here." Id. at 1217.

In Gilead, the Federal Circuit found that the district court had erroneously relied on the reasoning of two pre-Gilead decisions involving, as this case does, pre- and post-URAA patents. See id. at 1211. Plaintiffs rely on the same two cases here--Abbott Labs v. Lupin Limited, 2011 WL 1897322 (D. Del. May 19, 2011), and Brigham and Women's Hospital v. Teva Pharm. USA, Inc., 761 F. Supp. 2d 210 (D. Del. 2011). See Opp. at 17-18.

In Gilead, the Federal Circuit noted that in Ex Parte Pfizer, Inc., Patent Owner & Appellant, 2010 WL 532133 (Bd. Pat. App. & Interf. Feb. 12, 2010), the Board of Patent Appeals, on facts analogous to the facts of the instant case, found that the later-issued but earlier-expiring patent invalidated an earlier-issued later-expiring patent under the doctrine of obviousness-type double patenting because the later-expiring patent, in the Board's opinion, would impermissibly block the public from practicing the invention and obvious derivations of it disclosed in the patent that expired first. See Gilead, 253 F.3d at 1211 n.2. This

reference to Pfizer, as well as the other reasoning in Gilead, indicates that the Federal Circuit would in this case find the '471 patent obvious and invalid in view of the expired '444 patent.

If plaintiffs' position were correct, the public would be prevented from practicing the expired '444 patent and an obvious, patentably indistinct variation of it. This would violate "the "bedrock principle . . . that when a patent expires, the public is free to use not only the same invention claimed in the expired patent but also obvious or patentably indistinct modifications of that invention," id. at 1214, which is at the heart of the obviousness-type double patenting doctrine and which the Federal Circuit has found to be unaltered by the URAA.

The obviousness-type double patenting doctrine was well established when plaintiff applied for and when it accepted the '444 patent, which it knew would expire in 2011. Plaintiffs decided to take at least the risk that the '471 would be deemed invalid when the '444 expired. Infliximab was covered by the '471 genus patent, which plaintiff obtained, and by the '444 species patent that specifically claimed that antibody. As plaintiffs' counsel acknowledged at the August 16, 2016 hearing, such narrower patents are generally acquired to protect against claims of invalidity or infringement.[1] That risk was real here as the PTO has, in the

[1] Although not material to the analysis, I note that plaintiffs had a significant incentive to try to avoid the risk of invalidity of

pending reexamination, found that the '471 is obvious and invalid in view of two other patents plaintiffs held, U.S. Patent Nos. 5,656,272 and 5,698,195. See Final Rejection U.S. Pat. Reexam. No. 90/012,851, (Feb. 12, 2015); Advisory Action, U.S. Pat. Reexam. No. 90/012,851 (Apr. 29, 2015) (attached as Exs. 25, 26 to Defs' Stmnt.).

In Abbvie Inc. v. Mathilda & Terence Kennedy Inst. of Rheumatology Trust, 764 F.3d 1366, 1374 (Fed. Cir. 2014), the Federal Circuit confirmed that the doctrine of obviousness-type double patenting continues to apply where two patents that claim the same invention have different expiration dates. It reiterated the ruling of Gilead that if the later-expiring patent is merely an obvious variation of the invention disclosed and claimed in the reference patent, the later-expiring patent is invalid for obviousness-type double patenting. See id. at 1379. The reasoning of Abbvie is equally applicable to the facts of this case.

More specifically, the court finds that the expired '444 patent is a reference for the '471 Patent. The '471 patent is not patentably distinct from the '444 patent. Therefore, claims 1, 3, 5, 6, and 7 of the '471 patent are invalid. This conclusion is consistent with what is evidently the only other decision on

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the '471 patent by obtaining the '444 patent. As the parties agreed and informed the court, Remicade has generated sales in the United States of more than $4 billion a year.

comparable facts, MLC Intellectual Property v. Micron Tech., Inc., 2016 WL 4192009, at *3 n.4 (N.D. Cal. August 9, 2006).

IV. ORDER

In view of the foregoing, as ordered previously on August 19, 2016, Defendants' Motion for Summary Judgment of Invalidity of U.S. Patent No. 6,284,471 for Obviousness-Type Double Patenting (Docket No. 127) is ALLOWED.

UNITED STATES DISTRICT JUDGE

UNITED STATES DISTRICT COURT
DISTRICT OF MASSACHUSETTS

JANSSEN BIOTECH, INC. ET AL, Plaintiffs, v. CELLTRION HEALTHCARE CO. LTD., ET AL., Defendants	)	C.A. No. 15-10698-MLW

MEMORANDUM AND ORDER

WOLF, D.J. September 29, 2016

This Memorandum is based on the transcript of the decision rendered orally on August 18, 2016, allowing defendants Celltrion Healthcare Co., Ltd., Celltrion, Inc., and Hospira, Inc.'s, Motion For Summary Judgment Of Invalidity Of Claims 1, 3, 5, 6, 7 Of The '471 patent For Obviousness-Type Double Patenting In Light Of The Claims In The '195 And '272 patents (the "Reexam Motion"). The Memorandum adds citations and some text, and clarifies some language.

I. SUMMARY

Plaintiffs Janssen Biotech, Inc. and New York University, (collectively "Janssen"), are the holders of patents related to a biologic medication called Remicade, which is based on an antibody called infliximab. Plaintiffs allege that defendants Celltrion Healthcare Co., Ltd., Celltrion, Inc., and Hospira, Inc. (collectively "Celltrion") have infringed these patents by filing

an abbreviated Biologic License Application for a product that is "biosimilar" to Remicade, which is named Inflectra.

Defendants have moved for summary judgment of invalidity on one of the patents at issue in this case, U.S. Patent Number 6,284,471 (the "'471 patent" or "'471"). They argue that claims 1, 3, 5, 6 and 7 of the '471 patent are invalid under the doctrine of obviousness-type double patenting over the claims in two now-expired patent previously held by plaintiffs, U.S. Patent Nos. 5,656,272 (the "'272 patent") and 5,698,195 (the "'195 patent").

For the reasons explained in this Memorandum, the Reexam Motion is being allowed. In summary, the court finds that the '471 patent is not protected by the safe harbor provided by 35 U.S.C. §121. The court also finds that the one-way test for obviousness-type double patenting, rather than the two-way test, applies in this case. As the plaintiffs concede for present purposes, the claims in the '471 are obvious in view of the earlier-issued claims in the '195 and '272. Assuming, without finding, that the two-way obviousness test should be applied, there may be disputed facts concerning whether the United States Patent and Trademark Office (the "PTO") was solely responsible for any delay that caused the '195 patent and '272 patent to issue before the '471 patent. However, any such disputes would not be material because the claims of the '471 patent are also obvious under the two-way test.

II. PROCEDURAL HISTORY

On February 19, 2016, defendants filed their initial Motion for Partial Summary Judgment on the '471 patent (Docket No. 127) (the "Gilead Motion"), which plaintiffs opposed. The court subsequently allowed defendants to file a second motion for summary judgment, the Reexam Motion, which plaintiffs also opposed.

On August 16, 2016, the court heard oral argument on the Reexam Motion. On August 18, 2016, the court issued an oral decision allowing the Reexam Motion. On August 19, 2016, the court issued an order summarizing the reasons for that decision. Like the August 18, 2016 transcript, this Memorandum provides a fuller explanation of those reasons.

III. APPLICABLE STANDARDS

A. SUMMARY JUDGMENT

Federal Rule of Civil Procedure 56(a) provides that the court "shall grant summary judgment if the movant shows that there is no genuine dispute as to any material fact and the movant is entitled to judgment as a matter of law." A factual dispute, therefore, precludes summary judgment if it is "material" and "genuine." See Anderson v. Liberty Lobby, 477 U.S. 242, 247-48 (1986).

A fact is "material" if, in light of the relevant substantive law, "it has the potential of determining the outcome of the litigation." Maymi v. Puerto Rico Ports Auth., 515 F.3d 20, 25 (1st Cir. 2008); Martinez-Rodriguez v. Guevara, 597 F.3d 414, 419

(1st Cir. 2010). "Only disputes over facts that might affect the outcome of the suit under the governing law properly preclude the entry of summary judgment." Anderson, 477 U.S. at 248.

The party moving for summary judgment "bears the initial responsibility of informing the district court of the basis for its motion, and identifying those portions of [the record] which it believes demonstrate the absence of a genuine issue of material fact." Celotex Corp. v. Catrett, 477 U.S. 317, 323 (1986). However, the moving party's burden "may be discharged by 'showing' . . . that there is an absence of evidence to support the nonmoving party's case." Id. at 325. Summary judgment is, therefore, mandated "after adequate time for discovery and upon motion, against a party who fails to make a showing sufficient to establish the existence of an element essential to that party's case, and on which that party will bear the burden of proof at trial." Id. at 322; Gorski v. New Hampshire Dep't of Corr., 290 F.3d 466, 475-76 (1st Cir. 2002); Smith v. Stratus Computer, Inc., 40 F.3d 11, 12 (1st Cir. 1994).

B. OBVIOUSNESS-TYPE DOUBLE PATENTING

"An issued patent is presumed valid and can only be proven invalid by clear and convincing evidence." Biogen Idec MA Inc. v. Trustees of Columbia Univ. in City of New York, 332 F. Supp. 2d 286, 296 (D. Mass. 2004) (citing 35 U.S.C. §282).

> [35 U.S.C. §101] precludes more than one patent on the same invention . . . . Section 101, however, only prohibits a second patent on subject matter identical to an earlier patent. Thus, applicants can evade this statutory requirement by drafting claims that vary slightly from the earlier patent.
>
> [Therefore, courts have] fashioned a doctrine of nonstatutory double patenting (also known as "obviousness-type" double patenting) to prevent issuance of a patent on claims that are nearly identical to claims in an earlier patent. This doctrine prevents an applicant from extending patent protection for an invention beyond the statutory term by claiming a slight variant.

Id. (quoting Geneva Pharm., Inc. v. GlaxoSmithKline PLC, 349 F.3d 1373, 1377-78 (Fed. Cir. 2003)).

"'A later claim that is not patentably distinct from,' i.e., 'is obvious over[ ] or anticipated by,' an earlier claim is invalid for obviousness-type double patenting." Sun Pharm. Indus., Ltd. v. Eli Lilly & Co., 611 F.3d 1381, 1385 (Fed. Cir. 2010) (quoting Eli Lilly v. Barr Labs, 251 F.3d 955, 968 (Fed. Cir. 2001)). The doctrine of obviousness-type double patenting is primarily intended "to prevent unjustified timewise extension of the right to exclude granted by a patent no matter how the extension is brought about." In re Hubbell, 709 F.3d 1140, 1145 (Fed. Cir. 2013) (quoting In re Van Ornum, 686 F.2d 937, 943 (C.C.P.A. 1982)). "The obviousness-type double patenting analysis involves two steps." Abbvie Inc. v. Mathilda and Terence Kennedy Institute of Rheumatology Trust, 764 F.3d 1366, 1374 (Fed Cir. 2014).

> "First, the court 'construes the claim[s] in the earlier patent and the claim [s] in the later patent and determines the differences.' Second, the court 'determines whether those differences render the claims patentably distinct.'" Sun Pharm. Indus., Ltd. v. Eli Lilly & Co., 611 F.3d 1381, 1385 (Fed. Cir. 2010) (alteration in original) (quoting Pfizer, Inc. v. Teva Pharm. USA, Inc., 518 F.3d 1353, 1363 (Fed. Cir. 2008)). "'A later claim that is not patentably distinct from,' i.e., 'is obvious over[ ] or anticipated by, 'an earlier claim is invalid for obviousness-type double patenting." Id. at 1385 (alteration in original) (quoting Eli Lilly, 251 F.3d at 968).

Id.

IV. FACTS

The following undisputed facts are among those that are relevant.

On February 2, 1993, plaintiffs filed U.S. Pat. App. No. 08/013,413 (the "'413 Application"), which is a parent application to the '471, '195, and '272 patents. See Pltfs' Resp. Stmnt ¶5. During prosecution of the '413 Application, the PTO Examiner issued a restriction requirement that, among other things, required plaintiffs to separate into different applications claims relating to the "chimeric antibodies" and claims relating to methods for using the antibodies to treat various conditions. See Pltfs' Resp. Stmnt ¶9.

On February 4, 1994, plaintiffs filed the application that resulted in the '471 patent, U.S. Pat. App. No. 08/192,093 (the "'093 Application"). See id. ¶6. The '093 Application was labeled a continuation-in-part of both the '413 Application and another

application because it added new material not present in the '413 Application. See id.; Pltfs' Stmnt. ¶¶90-95. The '471 patent was issued on September 4, 2001 and expires on September 4, 2018. See Pltfs' Resp. Stmnt ¶6.

On February 4, 1994, plaintiffs also filed the application that led to the '272 patent, U.S. Pat. App. No. 08/192/102 (the "'102 Application"). See id. ¶7. This application was also a continuation-in-part of the same applications as the '471 patent. See id. The '272 patent was issued on August 12, 1997 and expired on August 12, 2014. See id.

On October 18, 1994, plaintiffs filed the application that led to the '195 patent, U.S. Pat. App. No. 08/324,799. See id. ¶8. This application was a continuation-in-part of the '093 Application and two other applications. See id. The '195 patent was issued on December 16, 1997 and expired on December 16, 2014. See id.

The '471 patent claims a group of "chimeric antibodies." See Pltfs' Resp. Stmnt. ¶11. Claims 1, 3 5, 6, and 7 encompass the infliximab antibody, which is also called "cA2." See id. ¶13. The '471 patent does not claim infliximab specifically. See '471 patent col.20; see id. col.14 l.57-col.15 l.8. However, the specification describes the infliximab antibody and refers to it as a preferred embodiment of the invention. See id.

The '195 patent "claims methods of using cA2, i.e., infliximab, for treatment of rheumatoid arthritis." See Pltfs' Resp. Stmnt ¶19. In particular, Claim 6 recites "[a] method of treating rheumatoid arthritis in a human comprising administering to the human an effective TNF-inhibiting amount of chimeric anti-TNF anti[b]ody cA2." Id.; '195 patent col.108 ll.57-59.

The '272 patent "claims methods of using cA2, i.e., infliximab, for treatment of TNFα-mediated Crohn's disease." Id. ¶20. In particular, Claim 7 recites "[a] method of treating TNFα-mediated Crohn's disease in a human comprising administering to the human an effective TNF-inhibiting amount of chimeric anti-TNF antibody cA2." Id.; '272 patent col.98 ll.13-15.

V. DISCUSSION

Defendants appropriately framed the three questions of particular importance to the Reexam Motion. They are:

1. Are the plaintiffs entitled to the statutory 35 U.S.C. §121 safe harbor? The court finds plaintiffs are not.
2. If the §121 safe harbor is not applicable, which test for obviousness applies, the one-way test or the two-way test? The court finds that the one-way test applies in this case.
3. If the two-way test applies, is it proper to consider the '471 patent specification when conducting the analysis, and if so, is the '471 patent is invalid? The

court finds that it is proper to consider the specification and, when that is done, the '471 patent is invalid under the two-way test.

35 U.S.C. §121 establishes a safe harbor against double patenting invalidation for certain patents issued based on applications that are divisional of earlier applications. See Pfizer, Inc. v. Teva Pharm. USA, Inc., 518 F.3d 1353, 1359 (Fed. Cir. 2008). The statute provides that:

> If two or more independent and distinct inventions are claimed in one application, the Director may require the application to be restricted to one of the inventions. If the other invention is made the subject of a divisional application which complies with the requirements of section 120 it shall be entitled to the benefit of the filing date of the original application. A patent issuing on an application with respect to which a requirement for restriction under this section has been made, or on an application filed as a result of such a requirement, shall not be used as a reference either in the Patent and Trademark Office or in the courts against a divisional application or against the original application or any patent issued on either of them, if the divisional application is filed before the issuance of the patent on the other application. The validity of a patent shall not be questioned for failure of the Director to require the application to be restricted to one invention.

35 U.S.C. §121 (emphasis added).

The Federal Circuit applies "'a strict test' for application of section 121, '[g]iven the potential windfall [a] patent term extension could provide to a patentee.'" G.D. Searle LLC v. Lupin Pharm., Inc., 790 F.3d 1349, 1354 (Fed. Cir. 2015) (quoting Geneva, 349 F.3d at 1382). After filing an original parent application,

an applicant can file subsequent continuing applications that claim the priority date of the parent. See 37 CFR ¶1.78. There are multiple types of subsequent applications that may be filed, including divisional applications and continuations-in-part ("CIPs"). See id. The §121 safe harbor applies only to a patent derived from an application that is labeled "divisional."

In Pfizer, the Federal Circuit considered the question of whether the §121 safe harbor applied to a patent resulting from an application labeled "continuation-in-part" and not "divisional." The district court had held that the '068 patent, which was filed as a CIP, could not be invalidated for double patenting by the '165 patent because both "derived from applications filed in response to the restriction requirement made in the common parent application." 518 F.3d at 1358. The Federal Circuit reversed, holding that "the protection afforded by section 121 to applications (or patents issued therefrom) filed as a result of a restriction requirement is limited to divisional applications." Id. at 1362.

In reaching its conclusion, the Federal Circuit rejected the argument that "the terms 'divisional' and 'continuation-in-part' are merely labels used for administrative convenience" and that "the term 'divisional application' as it is used in section 121 refers broadly to any type of continuing application filed as a result of a restriction, regardless of whether it is labeled by

the PTO, for administrative purposes, as a divisional, a continuation, or a CIP." Id. at 1360. The court reviewed the legislative history of §121, noting that prior to its passage "no protection was afforded to patent applications filed as a result of a restriction requirement . . . and such applications were often rejected or held invalid on double patenting grounds." Id. at 1360-61. It explained that Congress recognized that it was unfair to require an applicant to split out inventions into separate applications and to use those compelled separate applications to invalidate resulting patents. See id. at 1361. However, the court noted that "[t]here is no suggestion [] in the legislative history of section 121 that the safe-harbor provision was, or needed to be, directed at anything but divisional applications" and that "[t]he difference between divisional applications and CIPs . . . was well known at the time of the 1952 Patent Act." Id. at 1361-62. The Federal Circuit concluded that "[i]f the drafters wanted to include CIPs within the protection afforded by section 121, they could have easily done so." Id. at 1362. It, therefore, held that "the protection afforded by Section 121 to applications or patents issued therefrom filed as a result of a restriction requirement is limited to divisional applications." Id.

In Amgen Inc. v. F. Hoffman-La Roche Ltd, 580 F.3d 1340, 1353 (Fed. Cir. 2009), the Federal Circuit stated that "[w]e are persuaded by the reasoning in Pfizer that that the §121 safe harbor

provision does not protect continuation applications or patents descending from only continuation applications. The statute on its face applies only to divisional applications, and a continuation application, like a continuation-in-part application, is not a divisional application." It concluded that "[w]e decline to construe 'divisional application' in §121 to encompass Amgen's properly filed, properly designated continuation applications." Id. at 1354.

In Amgen, the Federal Circuit explained that:

> unlike a continuation-in-part application, a continuation application can satisfy the definition of a "divisional application" in MPEP §201.06. That is because a continuation-in-part application adds subject matter not disclosed in the earlier application, see MPEP §201.08, whereas continuation and divisional applications are limited to subject matter disclosed in the earlier application, see MPEP §§ 201.06, 201.07. This distinction, however, does not justify departing from a strict application of the plain language of §121, which affords its benefits to "divisional application[s]." See 35 U.S.C. § 121[.]

Id. at 1353.

In the instant case, the '471 patent resulted from an application that was properly labeled a continuation-in-part. As indicated earlier, Janssen added new material to the application resulting in the '471 patent that did not appear in the earlier '413 Application. The application in Amgen was a continuation application that, like a divisional application, did not add any new material. Therefore, Amgen considered an application that was

more factually analogous to a divisional application than the continuation-in-part in this case and nevertheless held that the §121 safe harbor did not apply because the application was labeled as a continuation and not a divisional.

The court understands that the PTO is now reexamining the '471 patent and has allowed an amendment to label the relevant application a divisional. However, this action is not final and, therefore, is not effective. See 37 CFR ¶1.530(k). Moreover, if any such amendment becomes final, the application will not be one filed before the issuance of the other relevant patents as required by §121. This issue was addressed in G.D. Searle, supra. There the Federal Circuit wrote, with regard to the patents at issue:

> The '113 application [for the '068 patent] cannot be a divisional of the '594 application, despite being designated as such in the reissue patent, because it contains new matter that was not present in the '594 application. Simply deleting that new matter from the reissued patent does not retroactively alter the nature of the '113 application . . . . [B]ecause neither of those applications is a division of the original '594 application, the Section 121 safe harbor does not apply to the RE '048 patents.

790 F.3d at 1354-55.

It is undisputed that the application leading to the '471 patent was filed as a continuation-in-part and not denominated a divisional. Nevertheless, Janssen argues that this court has the discretion to deem the '471 patent application divisional.

However, Amgen addressed and rejected a comparable argument, stating:

> Amgen has not presented us with any persuasive reason as to why we should deem the '178 and '179 continuation applications divisional applications for purposes of §121. Amgen does not dispute that it denominated the '178 and '179 applications continuations, that it checked the continuation application box on the submitted form, or that its applications met the PTO's definition of a continuation application in MPEP §201.07. See Amgen's Br. 38, 42. Instead, Amgen argues that, because the '178 and '179 continuation applications could have been filed as divisional applications, we should treat them as such for purposes of § 121. While this argument convinced the district court to regard the '178 and '179 continuation applications as divisional applications, we are not likewise convinced. We decline to construe "divisional application" in §121 to encompass Amgen's properly filed, properly designated continuation applications.

See 580 F.3d at 1354. Essentially, Pfizer, Amgen, and Searle established a bright line rule that only patents resulting from applications filed as divisional are protected by the §121 safe harbor. This court does not have the discretion to deem the '471 patent application divisional. Therefore, the '471 patent is not protected by the §121 safe harbor.

Accordingly, the court must decide whether the one-way test for determining obviousness double patenting or the unusual two-way test should apply. The defendants argue for the one-way test. The plaintiffs assert the two-way test applies.

The one-way test analyzes whether the claims of a challenged patent are obvious in light of the claims of an earlier-issued

reference patent. See Hubbell, 709 F.3d at 1149. Under the two-way test, the court conducts the one-way analysis and also considers "whether the [reference] patent claims are obvious over the [challenged patent] claims." Id. at 1149. "Thus, when the two-way test applies, some claims may be allowed that would have been rejected under the one-way test." In re Berg, 140 F.3d 1428, 1432 (Fed. Cir. 1998).

"The two-way test . . . is 'a narrow exception to the general rule of the one-way test . . . .'" Hubbell, 709 F.3d at 1149 (quoting Berg, 140 F.3d at 1432). "[T]he two-way test is appropriate only in the 'unusual circumstance' where 'the PTO is solely responsible for the delay in causing the second-filed application to issue prior to the first.'" Id. (quoting Berg, 140 F.3d at 1437). The "typical scenario" to which the two-way test is applied is "'when a later-filed improvement patent issues before an earlier filed basic invention.'" Berg, 140 F.3d at 1434 (quoting In re Braat, 937 F.2d 589, 593 (Fed. Cir. 1991)) (emphasis in original). "[B]asic and improvement patents should not be penalized by the rate of progress of the applications through the PTO, a matter over which the applicant does not have complete control." Braat, 937 F.2d at 593.

At the August 16, 2016 hearing, plaintiffs' counsel confirmed, at least for the purposes of the Reexam Motion, that the '471 patent is obvious for double patenting over the '195 patent or the '272

patent if the one-way test applies. See Aug. 16, 2016 Tr. at 160. This conclusion is correct.

The court finds that the one-way test is the appropriate test. The applications for the '471 and '272 patents' were both filed on February 4, 1994. As indicated earlier, "the two-way test [is] appropriate . . . in the unusual circumstance that the PTO is solely responsible for the delay in causing the second-filed application to issue prior to the first." Berg, 140 F.3d at 1437; see also Hubbell, 709 F.3d at 1149; In re Fallaux, 564 F.3d 1313, 1316 (Fed. Cir. 2009). In this case, the PTO did not decide the applications in reverse order of filing because the applications for the '471 patent and the '272 patent were filed the same day. Therefore, the language of Berg, Hubbell and Fallaux indicates that the two-way patenting test does not apply to those two patents at least.

The PTO's Manual of Patent Examining Procedures (the "MPEP") is consistent with this conclusion. It states that "[i]f both applications are filed on the same day, only a one-way determination of distinctness is needed in resolving the issue of double patenting." MPEP §804.II(B)(2)(b). The MPEP cites Berg for this proposition. Plaintiffs, however, argue that Berg does not control this question.

The court agrees that Berg does not control because the issue, although presented, was not decided. In Berg, the inventor chose

to file two applications simultaneously for inventions the Federal Circuit found could have been included in a single application. See 140 F.3d at 1433. The court held that "simultaneously filing two separate applications that could have been filed as one application disqualifies the applicant from the two-way test." Id. at 1435. The Federal Circuit neither accepted nor rejected the PTO's other reason for finding the two-way test inapplicable--the fact that the applications at issue were filed on the same day. See id.

In the instant case, in 1993, Janssen originally filed all of its claims in a single application. The PTO required that it file multiple applications, two of which were filed on the same day, February 4, 1994, as continuation-in-part applications. The third continuation-in-part application was filed on October 4, 1994. In Berg, the Federal Circuit addressed what should be done to avoid obviousness-type double patenting if the PTO determines that claims in a single application belong in multiple applications. It wrote that the inventor should file one or more divisional applications, which would be protected by the §121 safe harbor provision. See id. at 1435 to 36. Janssen did not do this.

In Berg, the applicant could have filed a single application. See Berg, 140 F.3d at 1435. Instead, it was "viewed as taking a calculated risk that, by simultaneously filing two separate applications, it might gain the advantage of a quickly issued narrow

patent and also the advantage of a broader application which took longer to issue as a patent but consequently had a later application date." Id. The Federal Circuit held that this disqualified Berg from receiving the benefit of the two-way test because "[e]ffectively extending the patent term [] is precisely the result that the doctrine of obviousness-type double patenting was created to prevent." Id.

In the instant case, the PTO's restriction requirement compelled the filing of a separate application for what became the '471 patent. However, plaintiff could have filed a divisional application. Instead, it chose to add new material to the application, perhaps strengthening the case for the issuance of the patent or making the PTO review process more protracted. This new application was properly labeled a continuation-in-part. Although plaintiffs conduct occurred before Berg was decided, the court concludes that they too took a calculated risk that disqualifies them from being eligible for employment of the two-way test.

As discussed earlier, the applications for the '272 patent and the '471 patent were filed on the same day. The '272 patent for a method of treating Crohn's disease with infliximab issued in 1997. The broader '471 genus patent issued in 2001. Janssen began receiving patent protection in 1997 under the '272 patent. If the obviousness double patenting doctrine were not applied here, Janssen's right to exclude the public from practicing the '272

patent would, in effect, be extended to 2018. As explained earlier, the primary justification for obviousness double patenting is "to prevent unjustified timewise extension of the right to exclude granted by a patent no matter how the extension is brought about." Hubbell, 709 F.3d at 1145. It is appropriate to apply this principle to find, as the PTO would according to the MPEP, that the usual one-way test is applicable in this case. As indicated earlier, plaintiffs concede for present purposes that the '471 patent is invalid for obviousness-type double patenting under this test.

However, in the interest of completeness, the court is also addressing the issue of how the two-way obviousness test would operate if it were applicable in this case. As indicated earlier, "the two-way test is appropriate only in the unusual circumstance that the PTO was solely responsible for the delay in causing the second-filed application to issue prior to the first." Berg, 140 F.3d at 1437. The Federal Circuit wrote in Hubbell that "obviousness-type double patenting is a question of law we review de novo. We review the Board's factual findings for substantial evidence." 709 F.3d at 1145. In the context of this case, as this statement indicates, there may be a genuine factual dispute concerning whether the PTO was solely responsible for the delay that caused the earlier-filed '471 to issue after the later-filed '195. See Engineered Prod. Co. v. Donaldson Co., 225 F. Supp. 2d

1069, 1089 (N.D. Iowa 2002), aff'd in part, rev'd in part and remanded, 147 F. App'x 979 (Fed. Cir. 2005).

If there was a genuine dispute concerning a fact that is material, the court would have a jury decide the disputed issue of whether the PTO was solely responsible for the delay that caused the later-filed application to result in the patent issued first. The jury's decision would determine whether the two-way test applies. Plaintiffs contend there are disputed facts and, therefore, summary judgment is not proper concerning the two-way test. However, even assuming without finding that whether the PTO was solely responsible for the delay is in genuine dispute, that fact is not material to the outcome of this motion for summary judgment because the plaintiffs would fail the two-way test.

With regard to the two-way test, one of the primary contested issues is whether the court may consider the '471 patent specification when deciding the second prong of the two-way test- - whether the claims of the '195 and '272 patents are obvious in light of the '471 patent. The court finds that the '471 patent specification may be considered.

"It is [] well settled that [the court] may look to a reference patent's disclosures of utility to determine the question of obviousness." Abbvie, 764 F.3d at 1381. "The Federal Circuit has repeatedly approved examination of the disclosed

utility of the invention claimed in an earlier patent to address the question of obviousness." Id. at 1382.

The Federal Circuit held in Sun that:

> the specification's disclosure may be used to determine whether a claim 'merely define[s] an obvious variation of what is earlier disclosed and claimed,' 'to learn the meaning of [claim] terms,' and to 'interpret [ ] the coverage of [a] claim.' . . . [W]here a patent features a claim directed to a compound, a court must consider the specification because the disclosed uses of the compound affect the scope of the claim for obviousness-type double patenting purposes.

611 F.3d at 1387 (citations omitted). In the instant case, it is undisputed that the '471 patent is to a compound. Therefore, it is appropriate to consider the specification.

Plaintiffs argue that Sun is restricted to circumstances in which the claims of the patent do not refer to a utility of the invention. However, the Federal Circuit in Sun rejected essentially the same argument, stating that "the holding of Geneva and Pfizer, that a 'claim to a method of using a composition is not patentably distinct from an earlier claim to the identical composition in a patent disclosing the identical use,' extends to any and all such uses disclosed in the specification of the earlier patent." Id. at 1385-86.

Here, the '471 patent's specification discloses the utility for the claimed genus of compounds, and the infliximab antibody in particular, for treating Crohn's disease and rheumatoid arthritis. The specification describes the infliximab antibody, see '471

patent col.20, even including it as a preferred embodiment of an invention, see id. col.14 l.57-col.15 l.8. It also discusses the utility of this class of compounds in treating various conditions, including rheumatoid arthritis and TNFα-mediated Crohn's disease. See Pltfs' Resp. Stmnt ¶21; '471 patent col.33 l.52 to col.35 l.3. The specification also describes two studies where the infliximab antibody was used to treat rheumatoid arthritis and Crohn's disease. See Pltfs' Reply Resp. Stmnt ¶¶36-37; '471 patent at col.58 l.23 to col.65 l.67, col.66 l.20 to col.68 l.20. Therefore, the '471 patent specification expressly describes the methods of using the infliximab antibody to treat Crohn's disease and rheumatoid arthritis that are claims in the '195 and '272 patents.

The Federal Circuit stated in Geneva Pharm., Inc. v. GlaxoSmithKline PLC, 349 F.3d 1373, 1385-86 (Fed. Cir. 2003), that a "claim to a method of using a composition is not patentably distinct from an earlier claim to the identical composition in a patent disclosing the identical use." Therefore, the '195 and '272 patents are obvious in light of the '471 patent under the second prong of the two-way obviousness test. As both prongs of the two-way obviousness test are satisfied, claims 1, 3, 5, 6, and 7 of the '471 patent would be invalid for obviousness-type double patenting if the two-way test were applicable.

Plaintiffs argue that, even if the court considers the '471 patent specification, disputed facts bar the court from concluding

on the motion for summary judgment that the '195 and '272 claims are obvious in light of the '471 patent. They assert that the '471 patent claims recited a genus of antibodies, not the infliximab antibody in particular. In contrast, the '195 and '272 patents recite methods for using the infliximab antibody itself. They contend that it is a disputed fact whether the methods of using a specific species in the genus are rendered obvious in light of the genus. This argument ignores the fact that the '471 patent specification expressly describes using the infliximab antibody itself to treat Crohn's disease and rheumatoid arthritis. The '471 does not limit its discussion to the more general question of using the genus to treat these diseases. Therefore, plaintiffs' argument that obviousness-type double patenting cannot be decided on a motion for summary judgment is not meritorious.

In summary, the court finds as follows. The '471 patent is not protected by the §121 safe harbor. The one-way test is applicable and, as plaintiffs concede for present purposes, the '471 patent fails that test. The court also finds that even if the two-way test were applicable, the '471 patent would be invalid for obviousness-type double patenting. Therefore, defendants' motion for summary judgment is being allowed.

VI. ORDER

In view of the foregoing, as previously ordered on August 19, 2016, Defendants' Motion For Summary Judgment Of Invalidity Of

Claims 1, 3, 5, 6, 7 Of The '471 patent For Obviousness-Type Double Patenting In Light Of The Claims In The '195 And '272 patents (Docket No. 176) is ALLOWED.

UNITED STATES DISTRICT JUDGE

UNITED STATES DISTRICT COURT
DISTRICT OF MASSACHUSETTS

JANSSEN BIOTECH, INC. ET AL, Plaintiffs,	)	
v.	)	C.A. No. 15-10698-MLW 16-11117-MLW
CELLTRION HEALTHCARE CO. INC., ET AL., Defendants.	)	

MEMORANDUM AND ORDER

WOLF, D.J. September 26, 2016

I. SUMMARY

In 2015, plaintiffs Janssen Biotech, Inc. and New York University (collectively "Janssen") filed Civil Action No. 15-10698 (the "2015 Action") against defendants Celltrion Healthcare Co., Ltd., Celltrion Inc., and Hospira, Inc. (collectively "Celltrion"). The case was brought pursuant to the Biologics Price Competition Act (the "BPCIA"), 42 U.S.C. §262, 35 U.S.C. §271(e)(2)(C). In the 2015 Action, Janssen alleged infringement by defendants' biosimilar product of several of Janssen's patents, including U.S. Patent No. 6,284,471 (the "'471" patent") and U.S. Patent No. 7,598,083 (the "'083" patent"), used to produce Remicade. Remicade is prescribed for chronic pain and generates billions of dollars of sales in the United States annually. In addition, in the 2015 Action, Janssen alleged violations of the BPCIA.

In 2016, Janssen filed Civil Action No. 16-11117 (the "2016 Action"), pursuant to 35 U.S.C. §271(a), alleging infringement of the '083 patent relating to Celltrion's activities outside the United States. The two cases have been consolidated.

In August 2016, the court decided two Celltrion motions for summary judgment and held that the '471 patent is invalid due to obviousness-type double patenting. Celltrion has moved for entry of a final judgment on this issue pursuant to Federal Rule of Civil Procedure 54(b). Janssen opposes that motion.

For the reasons explained in this Memorandum, Celltrion's motion for final judgment concerning the '471 patent is being allowed. The court has decided all issues concerning the validity of the '471 patent. Its judgment is, therefore, "final" for the purposes of Rule 54(b). In addition, there is no just reason to delay the entry of final judgment concerning the '471 patent. The decisions concerning the '471 patent are separable from the remaining issues in these cases. This court finds that the results of the pending reexamination of the '471 patent by the Patent and Trademark Office (the "PTO") will not affect the decision that the '471 patent is invalid. Nor will the Federal Circuit be required to decide the same issues more than once.

The equities also favor an immediate appeal of the decisions that the '471 patent is invalid. Uncertainty concerning that question could delay the sale of Inflectra in the United States.

Even if Inflectra is sold in the United States, uncertainty about whether Inflectra will be removed from the market because it infringes the '471 patent could discourage doctors from prescribing it. In either case, the individuals suffering great pain could be unfairly deprived for some time of a more affordable alternative to Remicade if the '471 patent is found on appeal to be invalid. In any event, legitimate price competition may be delayed while Janssen reaps substantial monopoly profits that would be unjustified if the '471 patent is invalid.

The BPCIA established an expedited procedure to promote the prompt resolution of claims of patent infringement by biosimilar products. This process is intended to reduce uncertainty and thus encourage the sale of non-infringing, more affordable biosimilars which may be important to human health. Granting Celltrion's Rule 54(b) motion serves the purposes of the BPCIA.

II. THE FACTS AND PROCEDURAL HISTORY

Janssen holds patents employed to create the biologic product "Remicade," which is based on the antibody infliximab. Remicade is used to treat intense, chronic pain. It can cost up to $20,000 per person a year. It generates revenue from sales in the United States of about $4 billion a year for Janssen and its parent, Johnson & Johnson.

The '471 patent is a patent to a group of chimeric antibodies including the infliximab antibody in Remicade. It is referred to

by the parties as the "antibody patent." The '083 patent claims the liquid solution in which cells are grown, such as the cells that produce the infliximab antibody. It is referred to by the parties as the "soup patent." In contrast to the '471 patent, the '083 patent does not include any reference to infliximab.

Celltrion has developed a product named Inflectra that is biosimilar to Remicade. Celltrion invested more than $100 million to develop Inflectra. Hospira, which is owned by Pfizer, has an exclusive agreement with Celltrion to market Inflectra in the United States.

In April 2016, the United States Food and Drug Administration approved Inflectra for sale. Pursuant to the BPCIA, Celltrion may begin selling Inflectra in the United States in October 2016. See Amgen Inc. v. Apotex Inc., 827 F.3d 1052, 1066 (Fed. Cir. 2016).

In essence, the BPCIA: requires the developer of a biosimilar product to disclose promptly the information necessary for a patent holder to decide whether it believes its patent has been infringed; requires the patent holder to sue promptly for any alleged infringement; and limits a patent holder to recovering a reasonable licensing fee, rather than lost profits, if the alleged infringement is not prosecuted promptly. See Amgen Inc. v. Sandoz Inc., 794 F.3d 1347, 1351-52 (Fed. Cir. 2015); 35 U.S.C. §271(e)(6). The BPCIA seeks to "'ensure that litigation surrounding relevant patents will be resolved expeditiously and

prior to the launch of the biosimilar product, providing certainty to the applicant, the reference product manufacturer, and the public at large.'" Amgen, 794 F.3d at 1363 (Newman, J. concurring in part, dissenting in part) (quoting Biologics and Biosimilars: Balancing Incentives for Innovation: Hearing Before the Subcommittee On Courts and Competition Policy of the House Committee On the Judiciary, 111th Cong. 9 (July 14, 2009) (statement of Rep. Eshoo)). The BPCIA is based in part on the understanding that uncertainty concerning whether an innovative biologic infringes a valid patent can discourage development and sale of a product that may be helpful to human health and more affordable than the patented product.

In these consolidated cases, Janssen now alleges that Inflectra infringes the '471 patent and the '083 patent.[1] In addition, Janssen asserts that Celltrion has not satisfied the requirements of the BPCIA. Janssen seeks declaratory and injunctive relief, and damages as well.

Despite being offered by the court several opportunities to do so, Janssen did not move for a preliminary injunction to prohibit the sale of Inflectra in the United States pending the outcome of these cases. Janssen did, however, move for a stay of

[1] In the 2015 Action, Janssen originally alleged infringement of six of its patents. Based on stipulations of dismissal of certain contentions, only the '471 patent and the '083 patent remain in dispute.

the litigation until the PTO concluded the pending reexamination of the '471 patent. Celltrion opposed that request.

At a May 19, 2016 hearing, the court denied the motion for a stay. See May 19, 2016 Transcript at 54-57. In explaining its decision, the court noted that the case was at an early stage and the litigation would be simplified at the district court level if the PTO found the '471 patent invalid. Id. The court cited these factors as favoring a stay. Id. at 54-55.

However, the court found these considerations to be outweighed by the undue prejudice to Celltrion and the public if the stay were granted. It reasoned, in part, that a stay would undermine a primary purpose of the BPCIA--achieving the expeditious resolution of the issues of alleged infringement to promote certainty as to whether a biosimilar product could be sold without exposing an innovator to the risk of an award of lost profits, which could be hundreds of millions, if not billions, of dollars in the instant cases. Id. at 55-56 (citing Amgen, 794 F.3d at 1363).

More specifically, the court stated that:

> A stay would expose the defendant to enormous potential damages for lost profits if it markets its biosimilar product and loses the case.
>
> That risk could deter the defendant from selling . . . a biosimilar that it is or may be entitled to sell without infringing the '471 patent. The PTO has so far for the purposes relevant to this case found the '471

> invalid [because it is] not eligible for the safe harbor provisions of the relevant statutes.
>
> If the defendants' biosimilar is delayed in getting to the market, the public will be deprived of a cost-effective alternative to Remicade.

Id. at 55. The court also noted that eliminating uncertainty was a reason for the creation of the declaratory judgment remedy and for establishing the Federal Circuit, citing its analysis in In re Columbia University Patent Litigation, 330 F. Supp. 2d 12, 17-18 & n.2 (D. Mass. 2004). The court concluded that the interest of minimizing uncertainty inherent is every patent case was particularly compelling in a case arising under the BPCIA, which was enacted to minimize uncertainty. See id. at 55.

The request for a stay having been denied, Celltrion filed two motions for summary judgment seeking declaratory judgments that the '471 patent is invalid because of obviousness-type double patenting. Janssen opposed the motions. The parties refer to the first motion as the "Gilead Motion" and the second motion as the "Reexam Motion." The court heard extensive arguments on those motions on August 16 and 17, 2016. For reasons described in detail orally, the court allowed Celltrion's motions and found the '471 patent invalid for obviousness-type double patenting.[2]

[2] In view of the appeal being authorized in this Memorandum and Order, the court will convert the transcripts of its oral decisions to more formal Memoranda and Orders.

On August 19, 2016, the court issued a written Order summarizing its reasons for granting the motions for summary judgment and finding the '471 patent invalid. With regard to Celltrion's first motion, the court wrote:

> Defendants' Motion for Summary Judgment of Invalidity of U.S. Patent No. 6,284,471 for Obviousness-Type Double Patenting (the "Gilead Motion") (Docket No. 127) is ALLOWED. Plaintiffs hold U.S. Patent No. 6,284,471 (the "'471 patent"). The '471 patent was issued on September 4, 2001. Standing alone, it would expire on September 4, 2018. Plaintiffs previously held U.S. Patent No. 6,790,444 (the "'444 Patent"). The '444 Patent was issued on September 14, 2004 and expired on July 11, 2011. The parties agree that the '471 patent is not patentably distinct from the '444 Patent. The Court of Appeals for the Federal Circuit held in Gilead Sciences., Inc. v. Natco Pharma Ltd., 753 F.3d 1208 (Fed. Cir. 2014), cert. denied, 135 S. Ct. 1530 (2015), that a later-issuing, earlier-expiring patent can act as a double-patenting reference for an earlier-issuing, later-expiring patent. The court finds that the reasoning in Gilead applies where, as here, the later-issued patent expires earlier because of the change to patent terms resulting from the Uruguay Round Agreements Act, codified at 35 U.S.C. §154. In essence, the court concludes that the statute was not intended to alter the judicial doctrine of obviousness double-patenting. See Gilead, 753 F.3d at 1216. Therefore, claims 1, 3, 5, 6, and 7 of the '471 patent are invalid for obviousness-type double-patenting in light of the patentably indistinct, earlier-expiring '444 Patent.

August 19, 2016 Order at ¶1.

With regard to Celltrion's second motion for summary judgment, the Reexam Motion, the court wrote in most pertinent part:

> The '471 patent is not entitled to the protection of the 35 U.S.C. §121 statutory safe harbor (the "Section 121 safe harbor"). The Section 121 safe harbor applies only

> to applications filed as "divisional." See Pfizer, Inc. v. Teva Pharm. USA, Inc., 518 F.3d 1353, 1362 (Fed. Cir. 2008). The '093 Application was filed as a continuation-in-part of U.S. Pat. App. No. 08/013,413 (the "'413 Application"). It was not filed as divisional of the '413 Application. The court does not have discretion to deem the '093 Application divisional. See id.; Amgen Inc. v. F. Hoffman-La Roche Ltd, 580 F.3d 1340, 1354 (Fed. Cir. 2009).

Id. at ¶2(b). The court found that the "one-way test" for obviousness was applicable to deciding the Reexam Motion and that the asserted claims of the '471 patent were obvious in light of claims in the two other patents relating to infliximab, U.S. Patent No. 5,698,195 and U.S. Patent No. 5,656,272. Id. at ¶2(c). The court also found that the '471 patent would fail the "two-way test" if it applied. Id. at ¶2(d).

Therefore, this court has decided all of the issues concerning the '471 patent. The remaining matters for the trial scheduled for February 2017 relate to the '083 "soup patent" and whether Celltrion satisfied the requirements of the BPCIA.

Following the court's decisions finding the '471 patent invalid, the parties made publicly reported statements. Janssen's announcement stated that it "is disappointed with the court's ruling and plans to appeal to the Court of Appeals for the Federal Circuit." See Press Release, Johnson & Johnson Announces Ruling Related to REMICADE in the District of Massachusetts Federal Court Hearing (Aug. 17, 2016) (attached as Ex. 2 to Celltrion's Memorandum in Support of the 54(b) Motion). Celltrion stated that

it is "committed to bringing biosimilars to patients in the U.S. as quickly as possible, and continuing with the preparation of our launch plans for Inflectra in 2016." Associated Press, Judge Invalidates Patent for Johnson & Johnson Rheumatoid Arthritis Drug, N.Y. Times (Aug. 17, 2016), http://www.nytimes.com/aponline/2016/08/17/us/apusjohnsonjohnsonpfizerremicade.html?emc=eta1.

As indicated earlier, Celltrion has moved for entry of partial final judgment concerning the '471 patent pursuant to Federal Rule of Civil Procedure 54(b), and Janssen has opposed that motion.

III. ANALYSIS

Rule 54(b) states that:

> When more than one claim for relief is presented in an action, whether as a claim, counterclaim, cross-claim, or third-party claim, or when multiple parties are involved, the court may direct the entry of a final judgment as to one or more but fewer than all of the claims or parties only upon an express determination that there is no just reason for delay and upon an express direction for the entry of judgment.

Fed. R. Civ. P. 54.

In deciding a motion under Rule 54(b), this "court must first determine that it is dealing with a 'final judgment.'" Curtiss-Wright Corp. v. General Elec. Co., 446 U.S. 1, 7 (1980). A "final" judgment is "an ultimate disposition of an individual claim entered in the course of a multiple claim action." Id. (internal quotation and citation omitted).

In this case, the court's two decisions granting Celltrion's motions for summary judgment finding the '471 patent invalid are final. Celltrion challenged the validity of the '471 patent in two ways in the Gilead and Reexam Motions for summary judgment. The court granted both motions. Doing so resolved all issues concerning the validity of the '471 patent. With regard to the '471 patent, there is nothing left for the court to do except enter judgment. It is, therefore, final. See Catlin v. United States, 324 U.S. 229, 233 (1945); W.L. Gore & Associates, Inc. v. Int'l Med. Prosthetics Research Associates, Inc., 975 F.2d 858, 863 (Fed. Cir. 1992).

Accordingly, the court must exercise sound judicial discretion in deciding whether to find that there is no just reason for delay and, therefore, to authorize an immediate appeal of its decisions finding the '471 patent invalid. See Curtiss-Wright, 446 U.S. at 8. In doing so, the court "must take into account judicial administrative interests as well as the equities involved." Id.

Janssen's claimed infringement of the '471 is separate and distinct from its remaining claims. A trial will be necessary to decide whether the '083 patent is valid and, if so, whether Inflectra infringes it. However, the '471 patent and the '083 patent relate to different technologies. As explained earlier, the '471 patent claims chimeric antibodies including infliximab.

In contrast, the '083 patent claims the liquid solution in which cells are grown, such as the cells that produce the infliximab antibody. The '471 and '083 patents involve distinctly different inventions. They present no common questions of fact or law concerning claim construction, invalidity, or infringement. Therefore, an immediate appeal concerning this court's final decisions concerning the '471 patent would not create the risk that the Federal Circuit will "have to decide the same issues more than once if there [is] a subsequent appeal" concerning the '083 patent. Id.

Similarly, Janssen's claim that Celltrion has not satisfied the requirements of the BPCIA is separate and distinct from its claim that the '471 patent is valid and is infringed by Inflectra. The alleged violation of the BPCIA presents issues of statutory interpretation and fact concerning Celltrion's conduct that are unrelated to the court's findings that the '471 patent is invalid under the doctrine of obviousness-type double patenting. Once again, any future appeal concerning the BPCIA issue will not require the Federal Circuit to decide the same issue more than once. Id.

Janssen has announced its intention to appeal. The issues now ripe for appeal will have to be decided by the Federal Circuit sooner or later. Therefore, authorizing an immediate appeal

concerning the '471 patent will also not require unnecessary effort by the Federal Circuit.

Janssen, however, argues that an immediate appeal of this court's decisions concerning the '471 patent will deprive it--and the courts--of the benefit of the PTO's reasoning and decision in the pending reexamination proceeding. This is essentially a reiteration of the argument made in support of Janssen's motion to stay, which the court found to be unmeritorious.

As indicated earlier, Remicade generates more than $4 billion a year in revenue for Janssen. Janssen, therefore, has a financial incentive to try to delay the appeal of the finding that the '471 patent is invalid in the hope that uncertainty concerning the infringement issue will contribute to a delay in the sale of Inflectra or discourage doctors from prescribing it.

While Janssen has a financial reason to prefer having the validity of the '471 patent litigated after the PTO renders its reexamination decision, the BPCIA required that it present its infringement claim promptly to this court. Janssen's decision not to seek an immediate appeal itself, and its opposition to Celltrion's Rule 54(b) motion, essentially reflect a disagreement with a primary purpose of the BPCIA--to expedite patent litigation concerning biosimilar products in order to maximize certainty, and diminish the risk that innovators will be unnecessarily deterred from offering those products to the public because of the threat

of liability for a patentee's lost profits. See Amgen, 794 F.3d at 1363 (Newman, J. concurring in part, dissenting in part).

Janssen contends that if it prevails in the PTO reexamination, this court's invalidity analysis would have to be reconsidered and reversed. As the court explained in granting Celltrion's Reexam Motion, this contention is incorrect. See August 18, 2016 Hearing Tr. at 14.

The Federal Circuit's decision in G.D. Searle LLC v. Lupin Pharm., Inc., 790 F.3d 1349 (Fed. Cir. 2015) rejected an identical argument relating to a reissue patent. In that case, the patent applicant filed a continuation-in-part application, the '113 application, as a result of a restriction requirement imposed by the PTO. Id. at 1352. The resulting '068 patent was later invalidated for obviousness-type double patenting by the Federal Circuit in Pfizer, Inc. v. Teva Pharmaceuticals USA, Inc., 518 F.3d 1353 (Fed. Cir. 2008), which found that the patent holder, Pfizer, was not entitled to protection under the §121 safe harbor because the '113 application was not filed as a divisional application. See id. Pfizer subsequently filed an application with the PTO for a reissue patent, arguing that the '113 application was improperly filed as a continuation-in-part instead of a divisional. See id. at 1353. The PTO allowed the amendment and issued the reissue patent with several changes including removing the new material that made the '113 application a

continuation-in-part and designating the '113 application as divisional. See id. Pfizer again sought protection under the §121 safe harbor for the reissue patent. See id. at 1354. The district court rejected Pfizer's argument. The Federal Circuit affirmed, stating in pertinent part:

> The '113 application for the '068 patent cannot be a divisional of the '594 application despite being designated as such in the reissue patent because it contains new matter that was not present in the '594 application. Simply deleting that new matter from the reissued patent does not retroactively alter the nature of the '113 application because neither of the '068 patent applications is a division of the original '594 application.

Id. at 1354-55.

Similarly, the application for the '471 patent was properly designated a continuation-in-part when it was filed because it contained new material. Under Searle, Janssen will not now be entitled to the protection of the §121 safe harbor even if the PTO retroactively characterizes the original application as divisional. Therefore, the PTO's decision will not affect the merit of this court's decisions that the '471 patent is invalid or be material to the Federal Circuit's review of them.

Accordingly, the court's decisions that the '471 patent is invalid are final and separable, and will not require duplication of effort or piecemeal consideration of related issues by the Federal Circuit. Nevertheless, the court recognizes that "[n]ot all final judgments on individual claims should be immediately

appealable even if they are in some sense separable from the remaining claims." Curtiss-Wright, 446 U.S. at 8. However, in this case the equities, including the public interest, favor authorizing an immediate appeal concerning the '471 patent.

As the seminal Curtiss-Wright case indicates, financial effects are among the equitable considerations a court may consider in deciding whether to authorize an appeal pursuant to Rule 54(b). Id. at 11 ("One of the equities which the District Judge considered was the difference between the statutory and market rates of interest."). In Curtiss-Wright, "the question before the District Court [] came down to which of the parties should get the benefit of the difference between the prejudgment and market rates of interest on debts admittedly owing and adjudged due while unrelated claims were litigated." Id. The Supreme court held that such financial consequences are cognizable in deciding a Rule 54(b) motion. Id. at 12-13.

In the instant cases, the risk of being required to pay Janssen for its lost profits if the '471 patent is valid and infringed could delay the sale of Inflectra in the United States. If so, Janssen would continue to have a monopoly generating more than $4 billion a year based on a patent this court has found to be invalid.

The court recognizes that Celltrion has said it is preparing to begin selling Inflectra in the United States in 2016. It is

not now certain whether it will do so. In any event, uncertainty relating to whether Inflectra infringes a valid '471 patent could affect decisions by potential investors in both Celltrion and Janssen. More significantly, uncertainty concerning whether Celltrion may be enjoined from selling Inflectra in the future could discourage doctors from prescribing it.

Most importantly, the public interest in making Inflectra available to doctors and their patients if it does not infringe a valid patent will be served by an immediate appeal. Remicade is prescribed for many people in great, chronic pain, which is why it generates billions in revenue annually. Remicade can cost up to $20,000 per patient per year. The cost may be unaffordable for some people. At a minimum, it makes their healthcare, and perhaps their insurance, more costly. A less expensive biosimilar alternative to compete fairly with Remicade would be in the public interest.

This public interest was recognized in the enactment of the BPCIA, with its expedited process for raising and resolving claims of infringement in cases involving biosimilar products. See Amgen, 794 F.3d at 1363 (Newman, J. concurring in part, dissenting in part). This court has given high priority to these consolidated cases in an effort to achieve the purposes of the BPCIA. Authorizing an appeal of the final decisions that the '471 patent

is invalid will further serve the purposes of the BPCIA. Therefore, Celltrion's Rule 54(b) Motion is being allowed.

IV. ORDER

In view of the foregoing, it is hereby ORDERED that:

1. Defendant's Motion for Entry of Final Judgment Pursuant to Federal Rule of Civil Procedure 54(b) Regarding U.S. Patent No. 6,284,471 (Docket No. 229) is ALLOWED.

2. A partial final judgment that U.S. Patent No. 6,284,471 is invalid shall enter for defendants as to Count 3 of Civil Action No. 15-10698-MLW

UNITED STATES DISTRICT JUDGE

US006284471B1

(12) **United States Patent**
Le et al.

(10) **Patent No.: US 6,284,471 B1**
(45) **Date of Patent: Sep. 4, 2001**

(54) **ANTI-TNFA ANTIBODIES AND ASSAYS EMPLOYING ANTI-TNFA ANTIBODIES**

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(73) Assignees: **New York University Medical Center**, New York, NY (US); **Centocor, Inc.**, Malvern, PA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **08/192,093**

(22) Filed: **Feb. 4, 1994**

Related U.S. Application Data

(63) Continuation-in-part of application No. 08/010,406, filed on Jan. 29, 1993, now abandoned, and a continuation-in-part of application No. 08/013,413, filed on Feb. 2, 1993, now abandoned, which is a continuation-in-part of application No. 07/943,852, filed on Sep. 11, 1992, now abandoned, which is a continuation-in-part of application No. 07/853,606, filed on Mar. 18, 1992, now abandoned, which is a continuation-in-part of application No. 07/670,827, filed on Mar. 18, 1991, now abandoned.

(51) **Int. Cl.**[7] **C07K 16/24**; A61K 39/395; C12N 15/00; G01N 33/53

(52) **U.S. Cl.** **435/7.1**; 435/69.6; 435/70.21; 530/387.3; 530/388.23; 530/391.3; 424/133.1; 424/139.1; 424/141.1

(58) **Field of Search** 530/387.3, 388.23, 530/391.3; 435/7.1, 240.27, 172.2, 70.21

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(57) **ABSTRACT**

Anti-TNF antibodies and anti-TNF peptides, specific for tumor necrosis factor (TNF) are useful for in vivo diagnosis and therapy of a number of TNF-mediated pathologies and conditions, as well as polynucleotides coding for anti-TNF murine and chimeric antibodies, peptides, methods of making and using the antibody or peptides in immunoassays and immuno-therapeutic approaches are provided, where the anti-TNF peptide is selected from a soluble portion of TNF receptor, an anti-TNF antibody or structural analog thereof.

9 Claims, 36 Drawing Sheets

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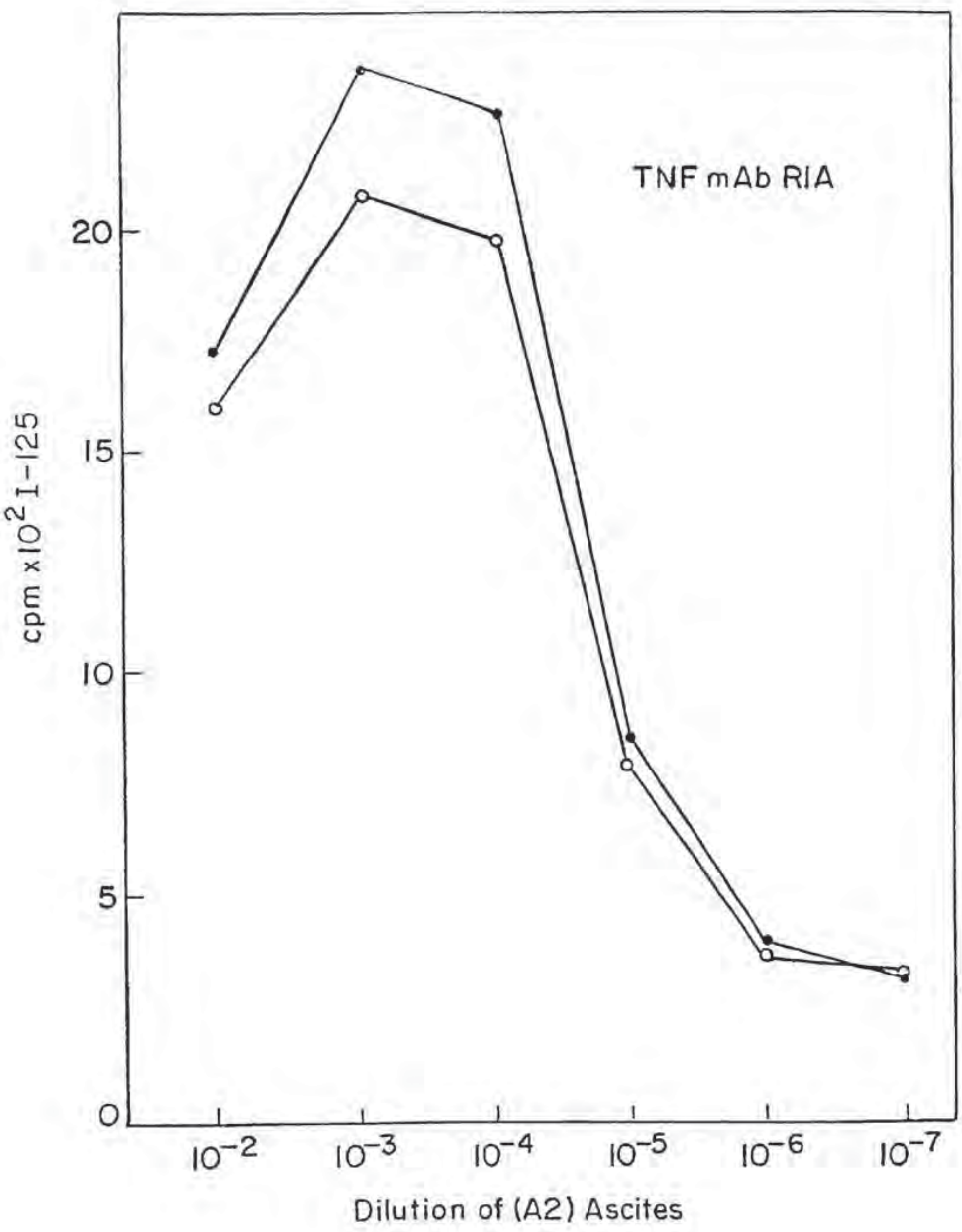

TNF mAb RIA
cpm x10² I-125
20
15
10
5
0
10⁻²
10⁻³
10⁻⁴
10⁻⁵
10⁻⁶
10⁻⁷
Dilution of (A2) Ascites

FIG. 1

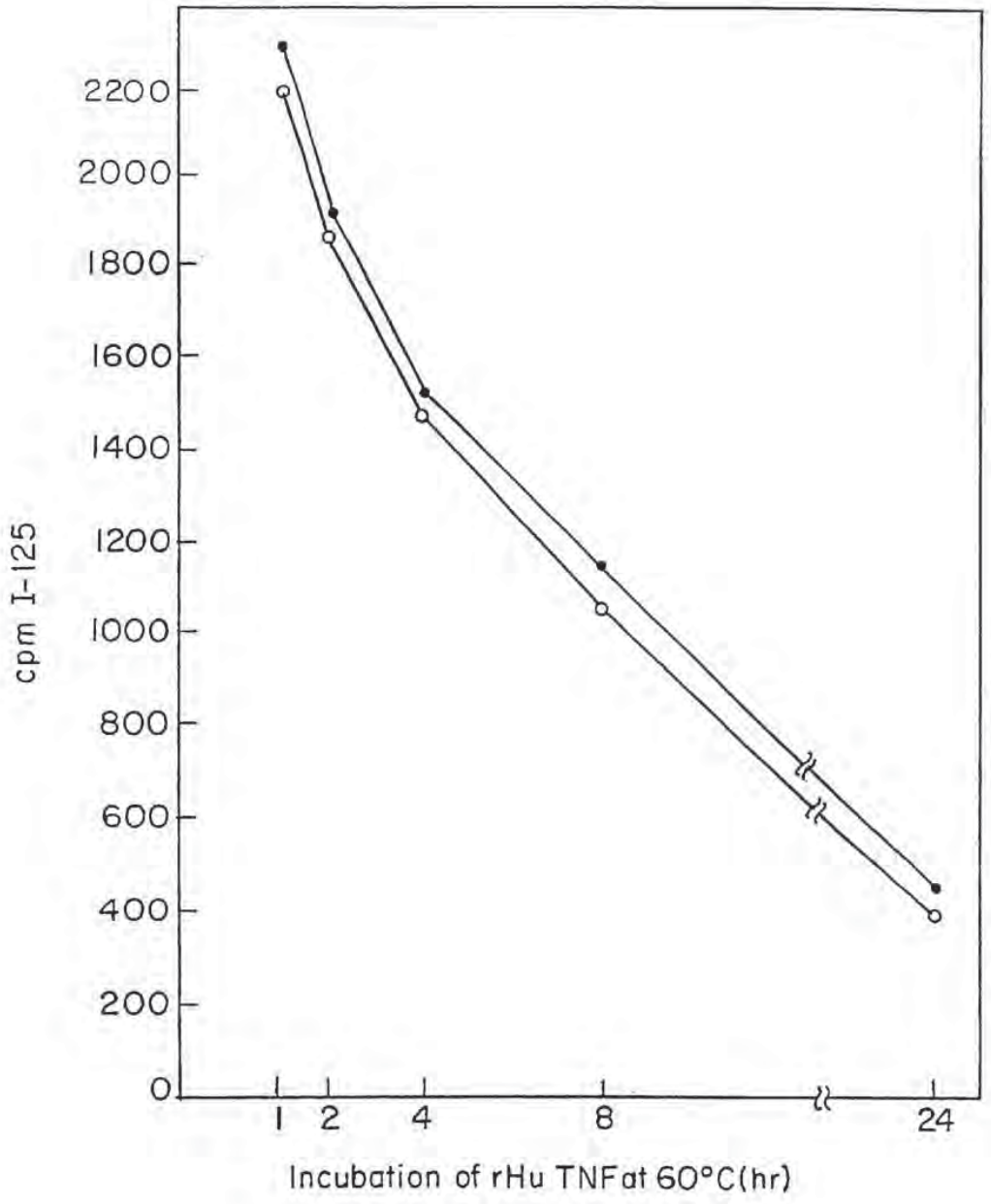

2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
cpm I-125
1
2
4
8
24
Incubation of rHu TNF at 60°C (hr)

FIG. 2

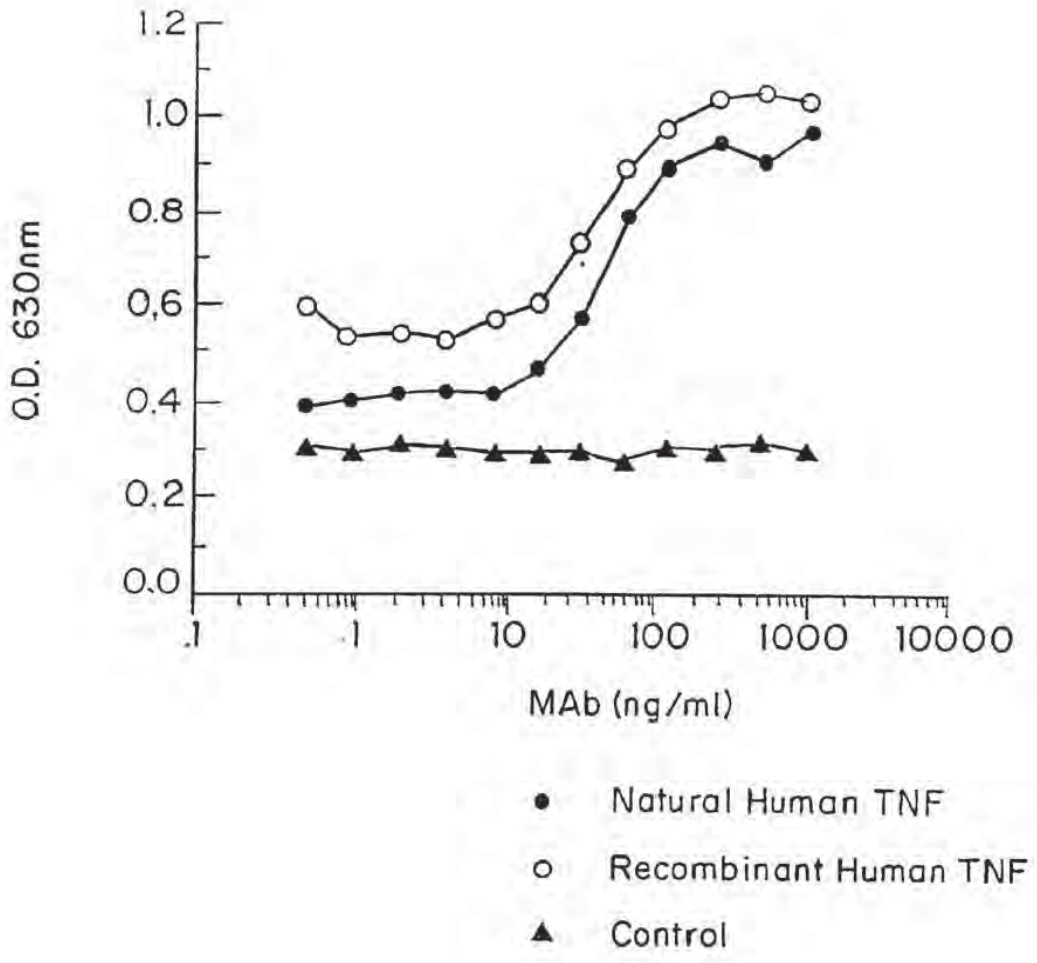

1.2
1.0
0.8
0.6
0.4
0.2
0.0
O.D. 630nm
.1
1
10
100
1000
10000
MAb (ng/ml)
Natural Human TNF
Recombinant Human TNF
Control

FIG. 3

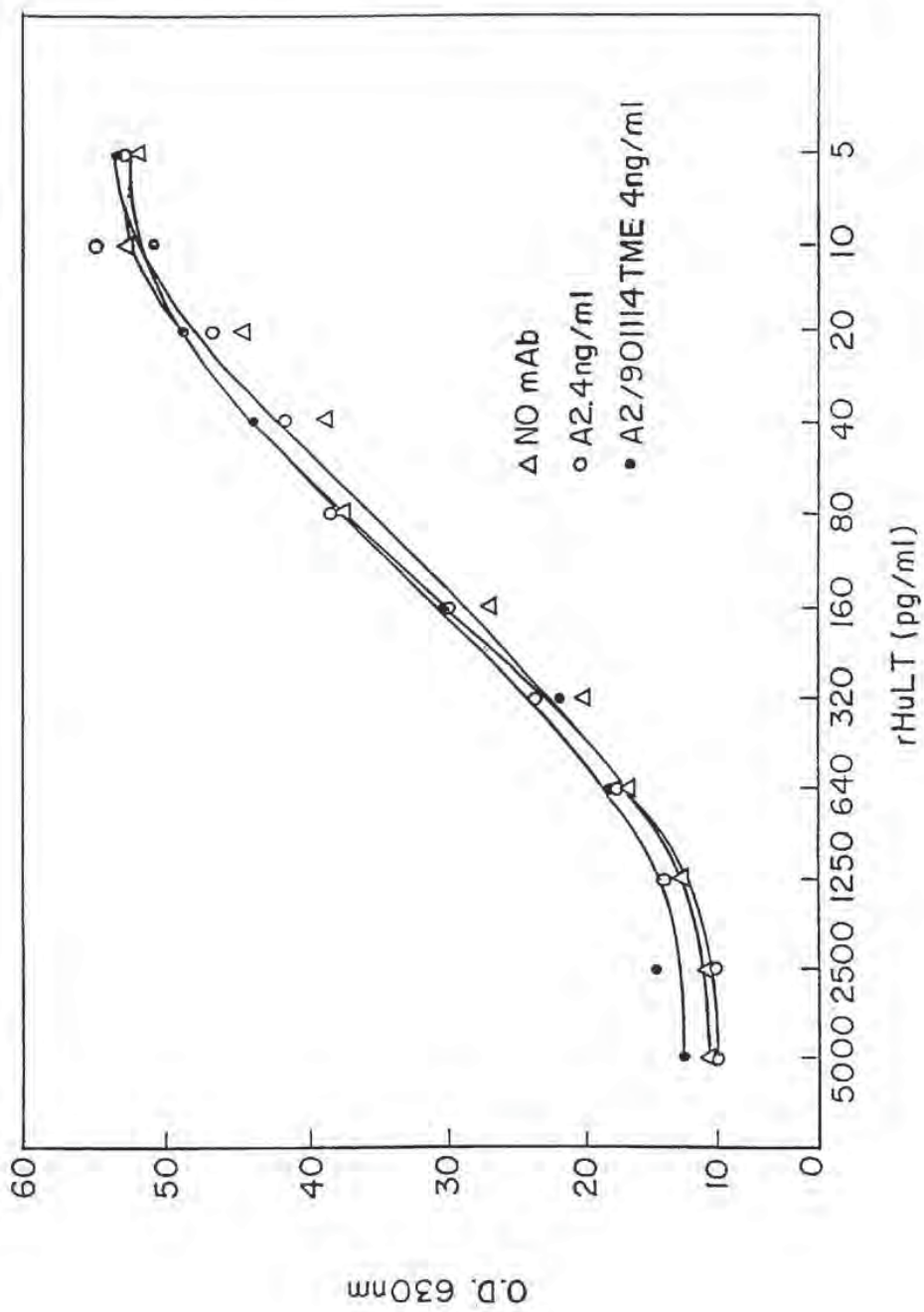

O.D. 630nm
0
10
20
30
40
50
60
5000
2500
1250
640
320
160
80
40
20
10
5
rHuLT (pg/ml)
Δ NO mAb
o A2.4ng/ml
• A2/9011114 TME 4ng/ml

FIG. 4

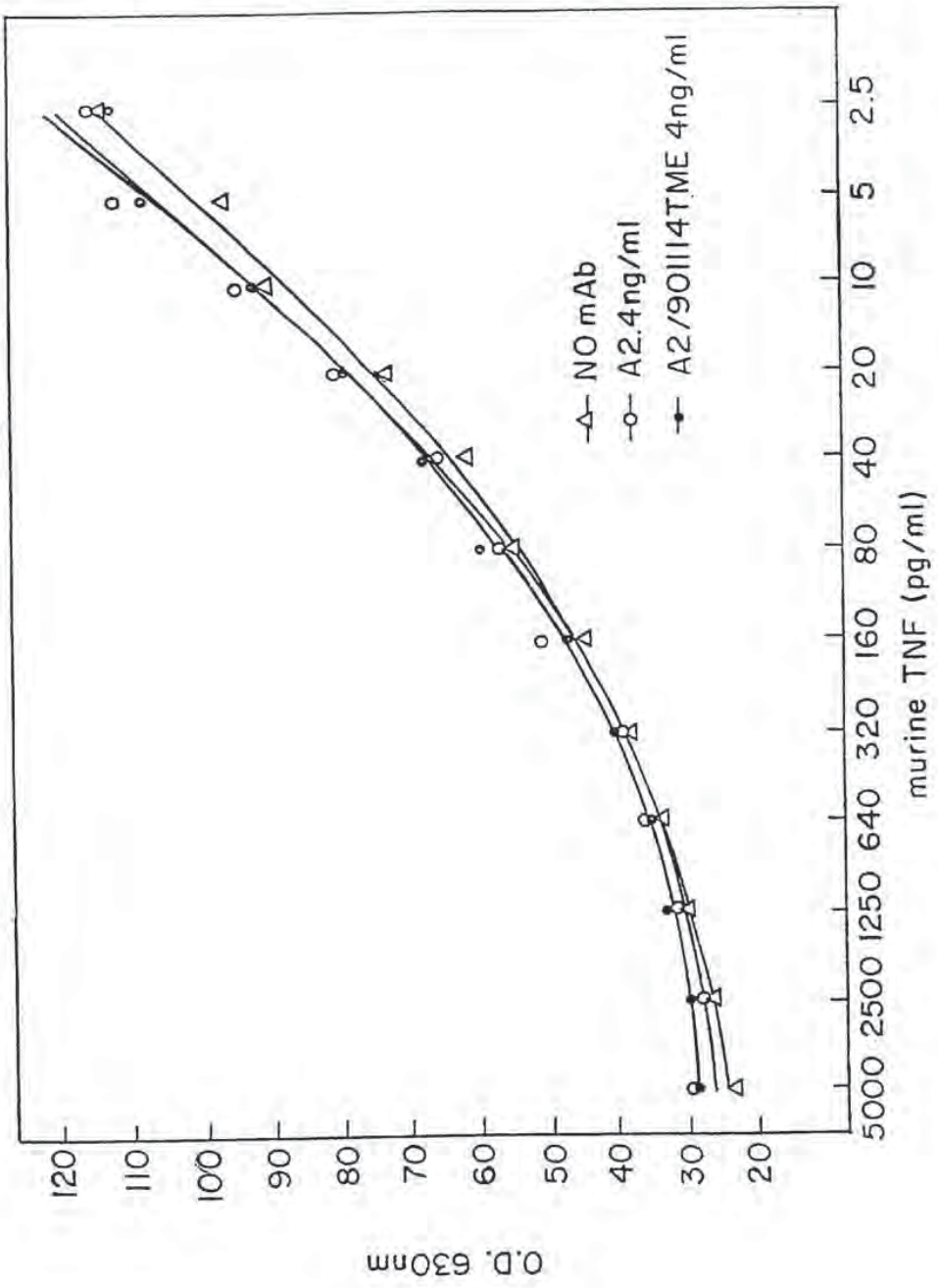

O.D. 630nm
120
110
100
90
80
70
60
50
40
30
20
5000 2500 1250 640 320 160 80 40 20 10 5 2.5
murine TNF (pg/ml)
NO mAb
A2.4ng/ml
A2/9011I4TME 4ng/ml

FIG. 5

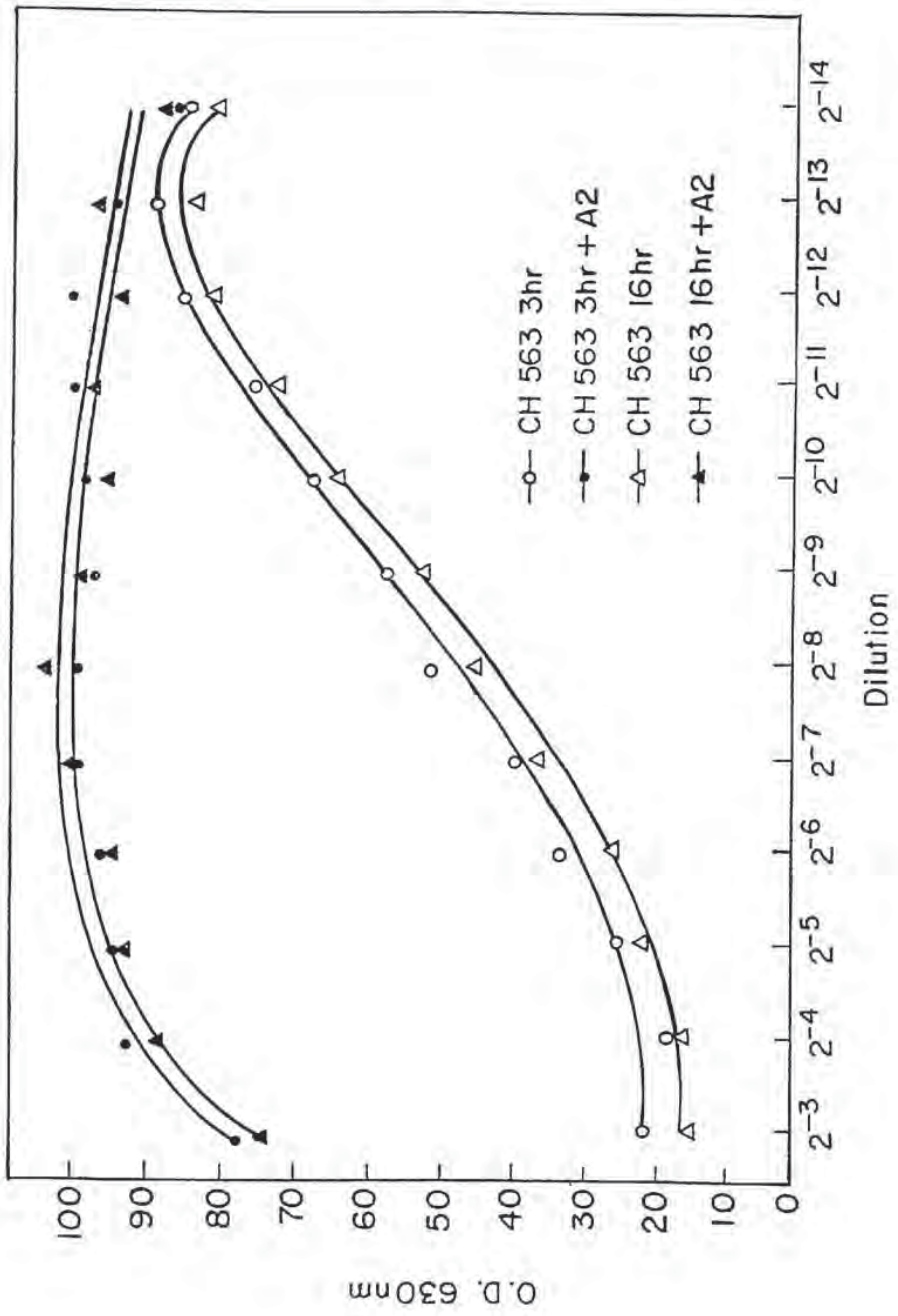

O.D. 630nm
100
90
80
70
60
50
40
30
20
10
0
2^{-3} 2^{-4} 2^{-5} 2^{-6} 2^{-7} 2^{-8} 2^{-9} 2^{-10} 2^{-11} 2^{-12} 2^{-13} 2^{-14}
Dilution
CH 563 3hr
CH 563 3hr + A2
CH 563 16hr
CH 563 16hr + A2

FIG. 6

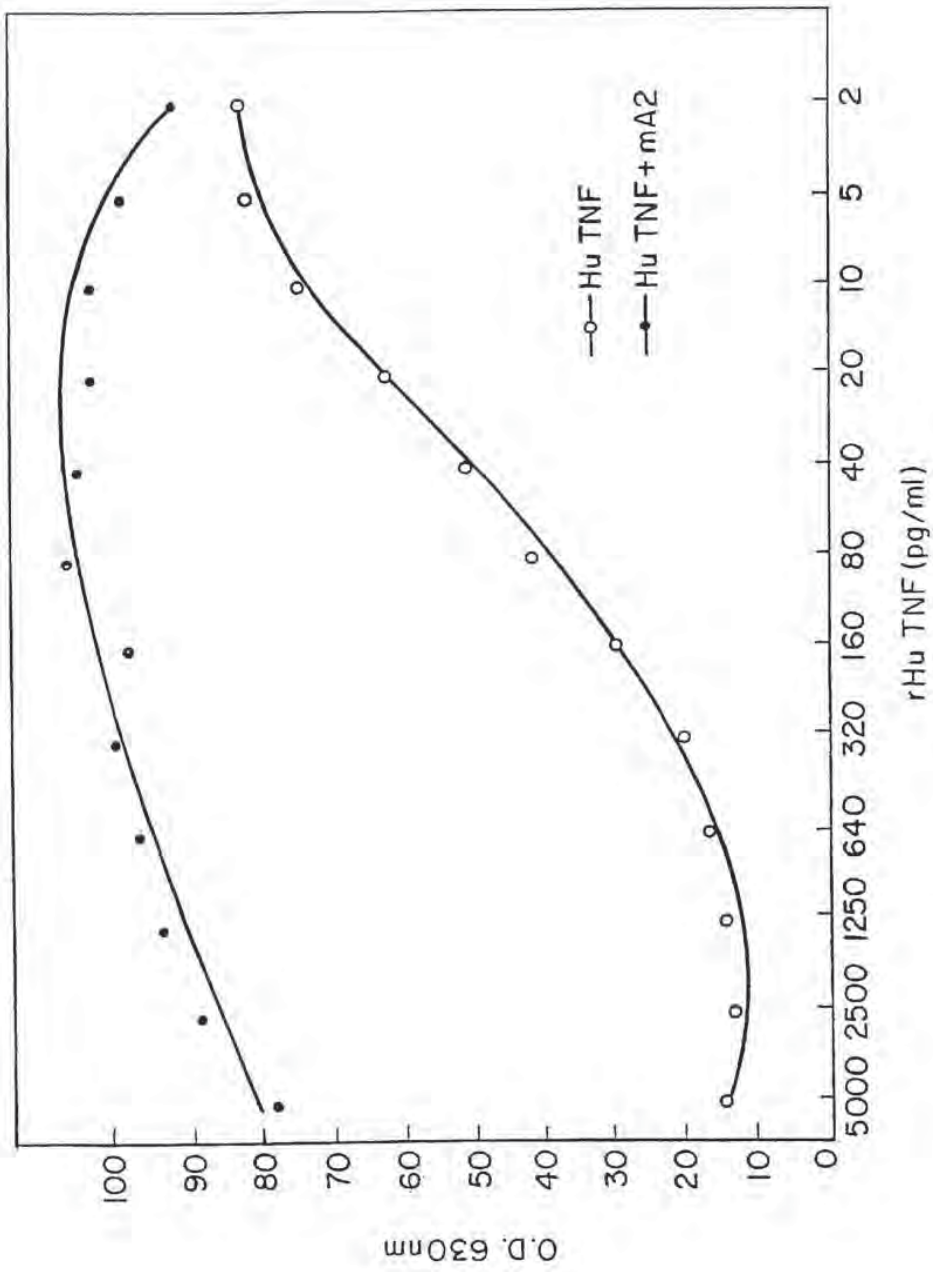
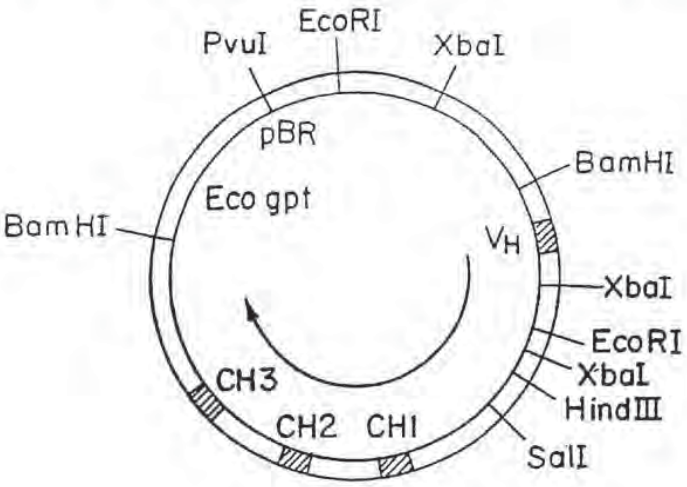
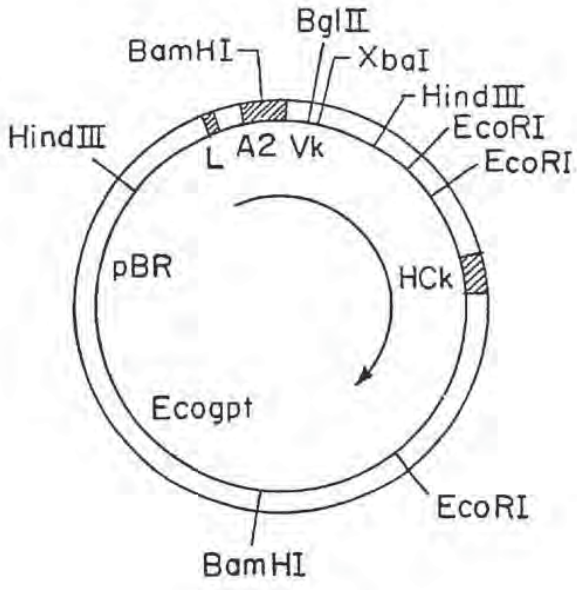

O.D. 630nm
0
10
20
30
40
50
60
70
80
90
100
0
2500
1250
640
320
160
80
40
20
10
5
2
rHu TNF (pg/ml)
Hu TNF
Hu TNF + mA2

FIG. 7



pA2HG1apgpt

FIG. 8A



pA2HuKapgpt

FIG. 8B

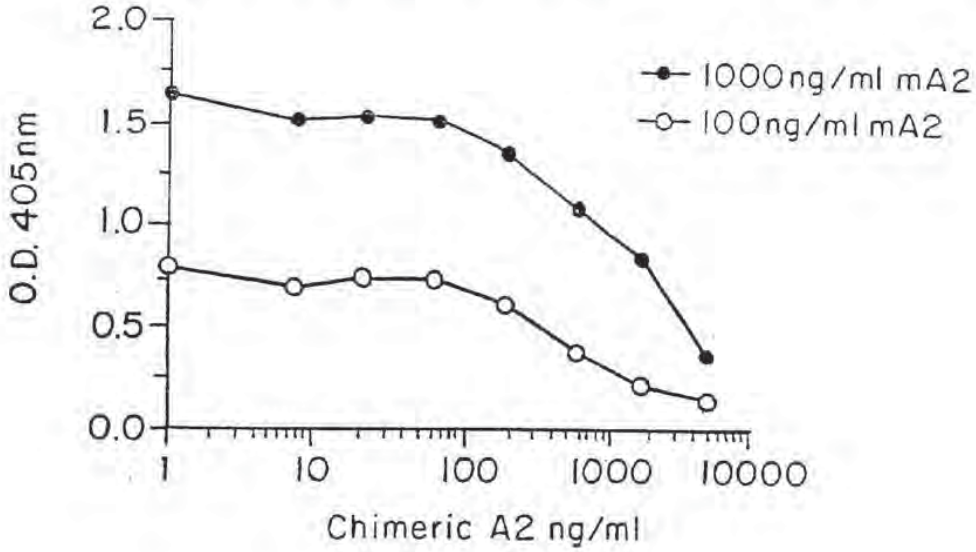

2.0
1.5
1.0
0.5
0.0
O.D. 405nm
1000ng/ml mA2
100ng/ml mA2
1
10
100
1000
10000
Chimeric A2 ng/ml

FIG. 9A

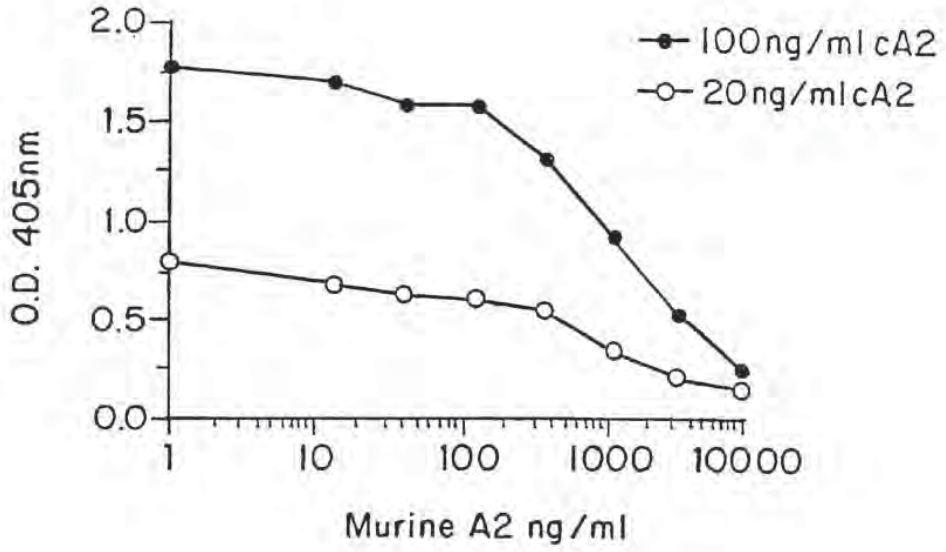

2.0
1.5
1.0
0.5
0.0
O.D. 405nm
100ng/ml cA2
20ng/ml cA2
1
10
100
1000
10000
Murine A2 ng/ml

FIG. 9B

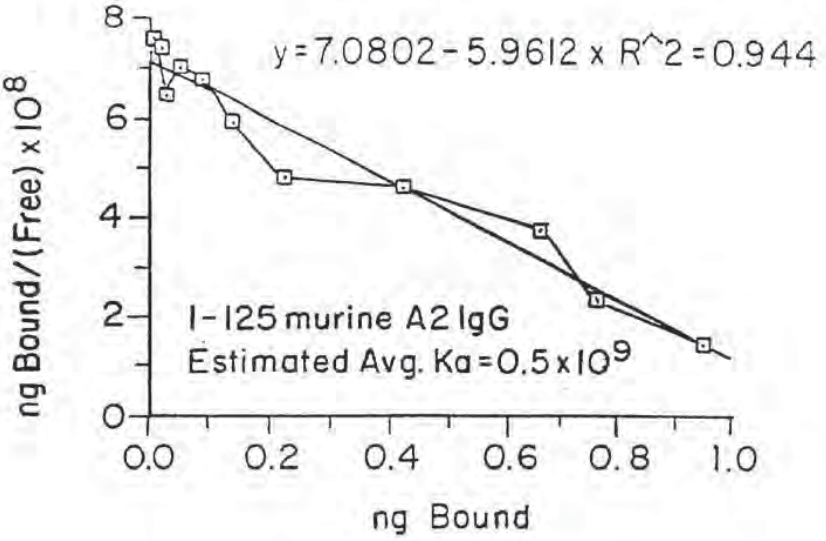


FIG. 10A

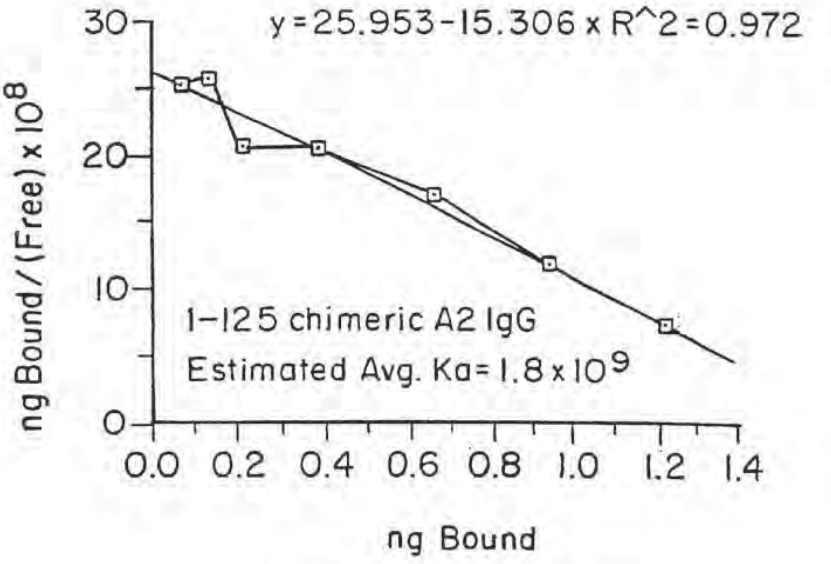


FIG. 10B

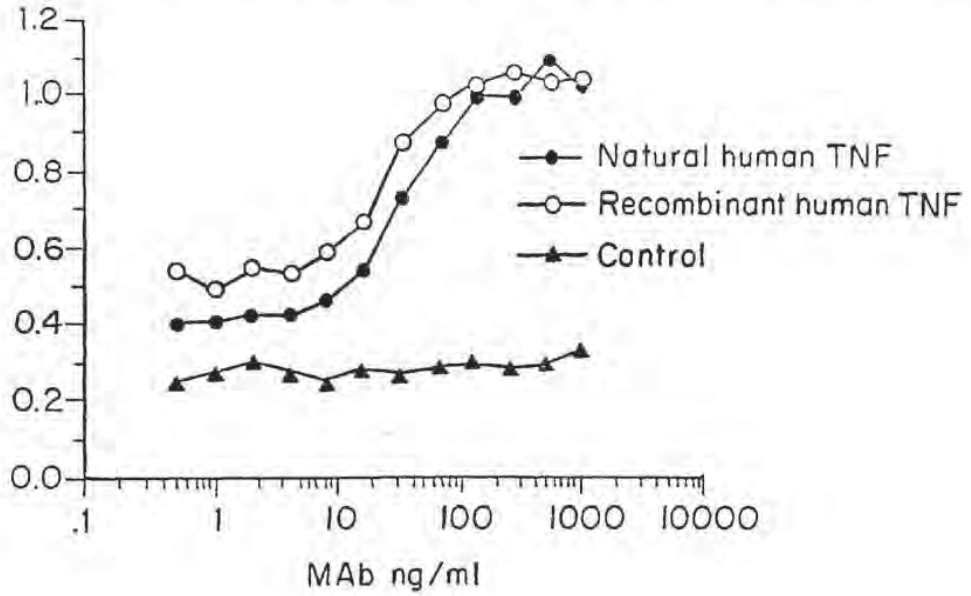

1.2
1.0
0.8
0.6
0.4
0.2
0.0
.1
1
10
100
1000
10000
MAb ng/ml
Natural human TNF
Recombinant human TNF
Control

FIG. 11

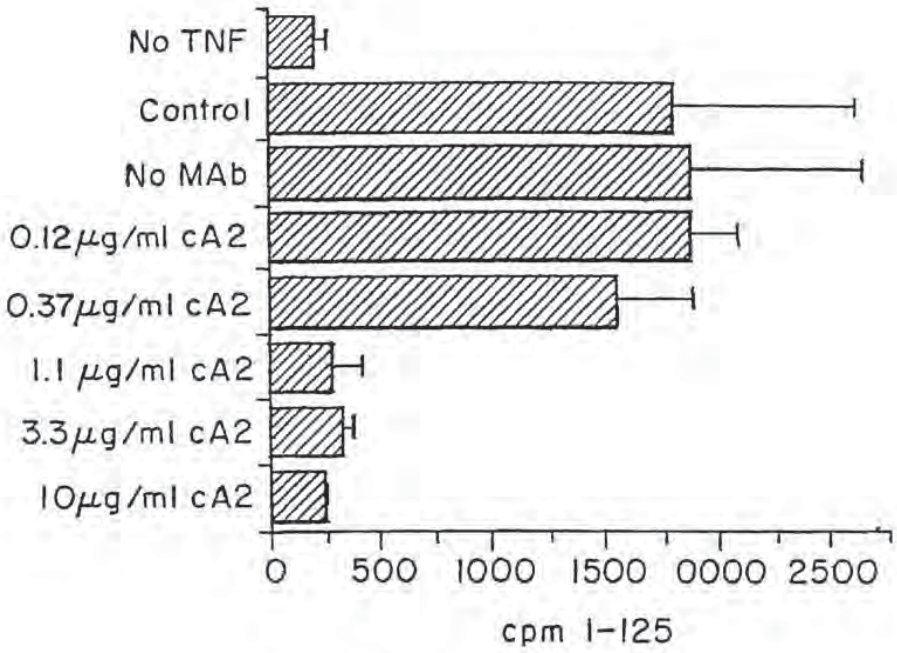

No TNF
Control
No MAb
0.12μg/ml cA2
0.37μg/ml cA2
1.1 μg/ml cA2
3.3μg/ml cA2
10μg/ml cA2
0
500
1000
1500
0000
2500
cpm I-125

FIG. 12

```
1                   10
Val Arg Ser Ser Ser Arg Thr Pro Ser Asp Lys Pro Val Ala His Val Val Ala Asn Pro

21                  30
Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg Ala Asn Ala Leu Leu Ala Asn Gly

41                  50
Val Glu Leu Arg Asp Asn Gln Leu Val Val Pro Ser Glu Gly Leu Tyr Leu Ile Tyr Ser

61                  70
Gln Val Leu Phe Lys Gly Gln Gly Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile

81                  90
Ser Arg Ile Ala Val Ser Tyr Gln Thr Lys Val Asn Leu Leu Ser Ala Ile Lys Ser Pro

101                 110
Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys Pro Trp Tyr Glu Pro Ile Tyr Leu

121                 130
Gly Gly Val Phe Gln Leu Glu Lys Gly Asp Arg Leu Ser Ala Glu Ile Asn Arg Pro Asp

141                 150
Tyr Leu Asp Phe Ala Glu Ser Gly Gln Val Tyr Phe Gly Ile Ile Ala Leu
```

FIG. 13

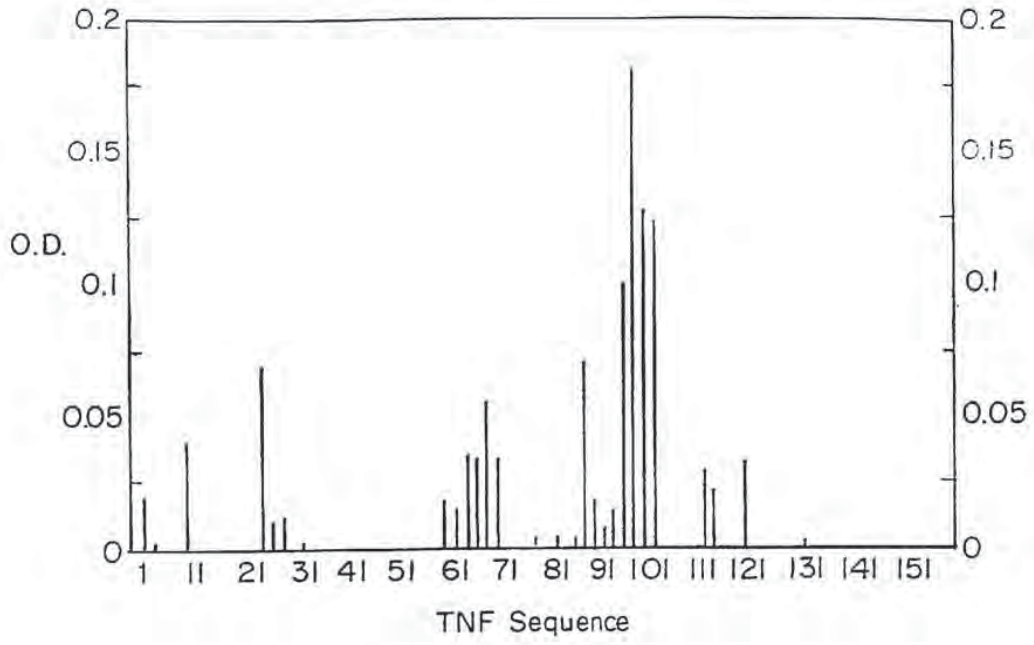


FIG. 14A

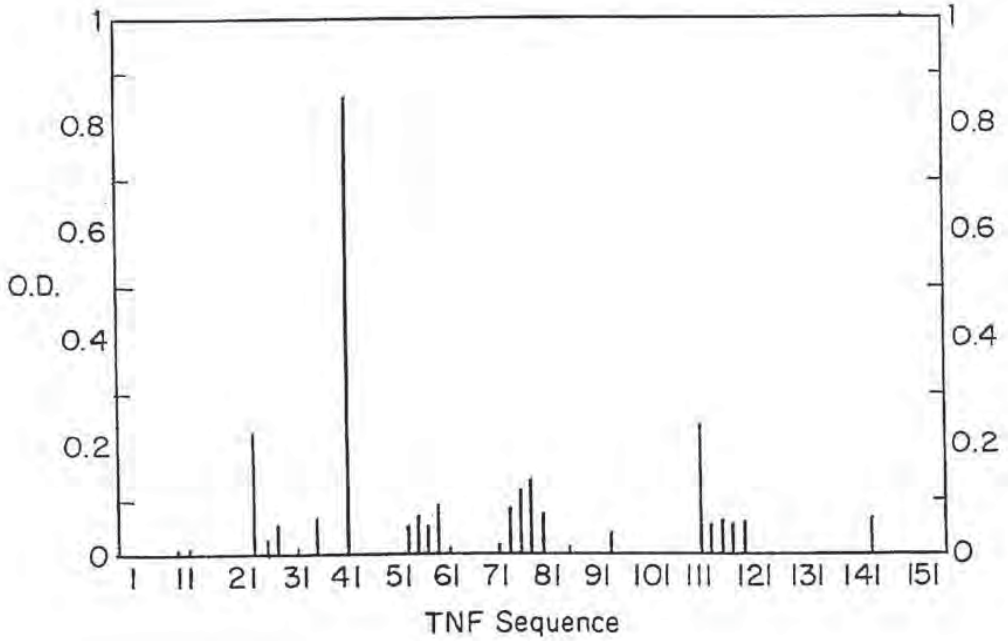


FIG. 14B

```
1   Val Arg Ser Ser Ser Arg Thr Pro Ser Asp Lys Pro Val Ala His Val Val Ala Asn Pro
                    10
21  Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg Ala Asn Ala Leu Leu Ala Asn Gly
                    30
41  Val Glu Leu Arg Asp Asn Gln Leu Val Val Pro Ser Glu Gly Leu Tyr Leu Ile Tyr Ser
                    50
61  Gln Val Leu Phe Lys Gly Gln Gly Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile
                    70
81  Ser Arg Ile Ala Val Ser Tyr Gln Thr Lys Val Asn Leu Leu Ser Ala Ile Lys Ser Pro
                    90
101 Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys Pro Trp Tyr Glu Pro Ile Tyr Leu
                   110
121 Gly Gly Val Phe Gln Leu Glu Lys Gly Asp Arg Leu Ser Ala Glu Ile Asn Arg Pro Asp
                   130
141 Tyr Leu Asp Phe Ala Glu Ser Gly Gln Val Tyr Phe Gly Ile Ile Ala Leu
                   150
```

FIG. 15

```
GACATCTTGCTGACTCAGTCTCCAGCCATCCTGTCTGTGAGTCCAGGAGAAAGAGTCAGT
AspIleLeuLeuThrGlnSerProAlaIleLeuSerValSerProGlyGluArgValSer

TTCTCCTGCAGGGCCAGTCAGTTCGTTGGCTCAAGCATCCACTGGTATCAGCAAAGAACA
PheSerCysArgAlaSerGlnPheValGlySerSerIleHisTrpTyrGlnGlnArgThr

AATGGTTCTCCAAGGCTTCTCATAAAGTATGCTTCTGAGTCTATGTCTGGGATCCCTTCC
AsnGlySerProArgLeuLeuIleLysTyrAlaSerGluSerMetSerGlyIleProSer

AGGTTTAGTGGCAGTGGATCAGGGACAGATTTTACTCTTAGCATCAACACTGTGGAGTCT
ArgPheSerGlySerGlySerGlyThrAspPheThrLeuSerIleAsnThrValGluSer

GAAGATATTGCAGATTATTACTGTCAAGAAAGTCATAGCTGGCCATTCACGTTCGGCTCG
GluAspIleAlaAspTyrTyrCysGlnGlnSerHisSerTrpProPheThrPheGlySer

GGGACAAAATTTGGAAGTAAAA
GlyThrAsnLeuGluValLys
```

FIG. 16A

GAAGTGAAGCTTGAGGAGTCTGGAGGAGGCTTGGTGCAACCTGGAGGATCCATGAAACTC
GluValLysLeuGluGluSerGlyGlyGlyLeuValGlnProGlyGlySerMetLysLeu

TCCTGTGTTGCCTCTGGATTCATTTTCAGTAACCACTGGATGAACTGGGTCCGCCAGTCT
SerCysValAlaSerGlyPheIlePheSerAsnHisTrpMetAsnTrpValArgGlnSer

CCAGAGAAGGGGCTTGAGTGGGTTGCTGAAATTAGATCAAAATCTATTAATTCTGCAACA
ProGluLysGlyLeuGluTrpValAlaGluIleArgSerLysSerIleAsnSerAlaThr

CATTATGCGGAGTCTGTGAAAGGGAGGTTCACCATCTCAAGAGATGATTCCAAAAGTGCT
HisTyrAlaGluSerValLysGlyArgPheThrIleSerArgAspAspSerLysSerAla

GTGTACCTGCAAATGACCGACTTAAGAACTGAAGACACTGGCGTTTATTACTGTTCCAGG
ValTyrLeuGlnMetThrAspLeuArgThrGluAspThrGlyValTyrTyrCysSerArg

AATTACTACGGTAGTACCTACGACTACTGGGGCCAAGGCACCACTCTCACAGTGTCC
AsnTyrTyrGlySerThrTyrAspTyrTrpGlyGlnGlyThrThrLeuThrValSer

FIG. 16B

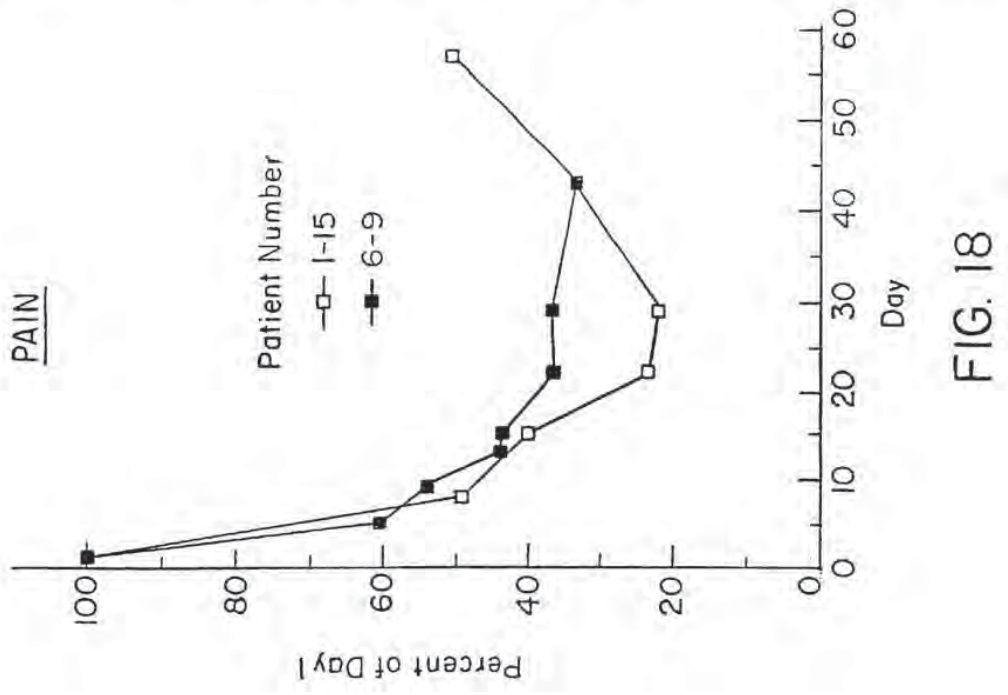

PAIN
Patient Number
1-15
6-9
Percent of Day 1
Day
0
20
40
60
80
100
0
10
20
30
40
50
60

FIG. 18

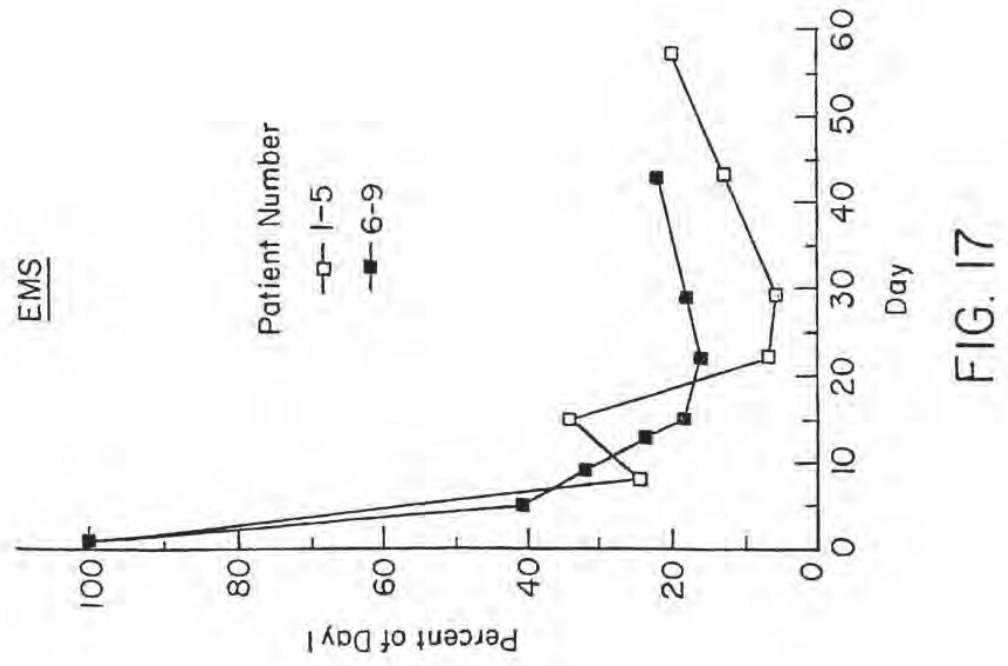

EMS
Patient Number
1-5
6-9
Percent of Day 1
Day
0
20
40
60
80
100
0
10
20
30
40
50
60

FIG. 17

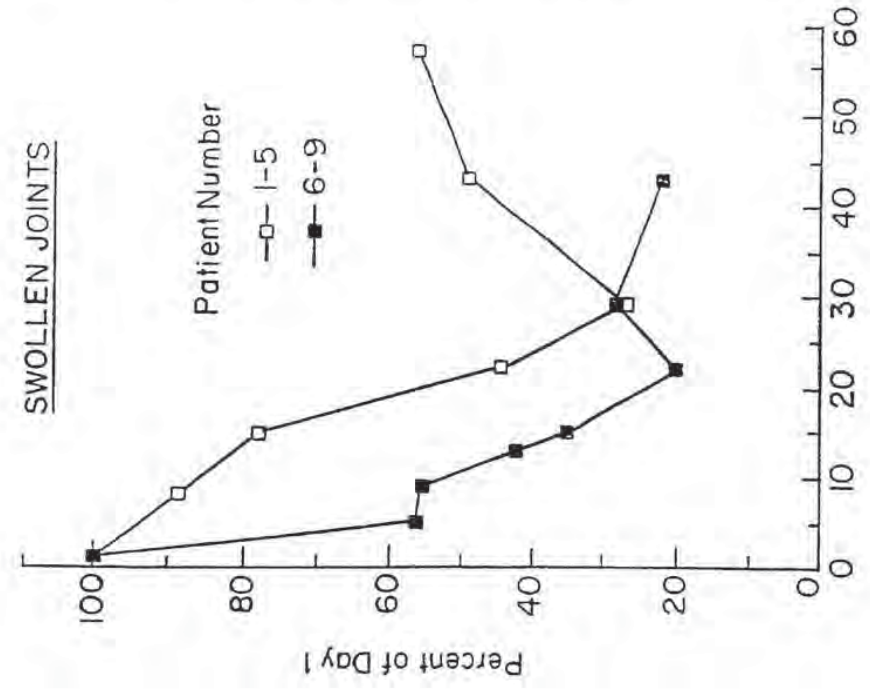

SWOLLEN JOINTS
Patient Number
1-5
6-9
Percent of Day 1
Day
0
20
40
60
80
100
0
10
20
30
40
50
60

FIG. 20

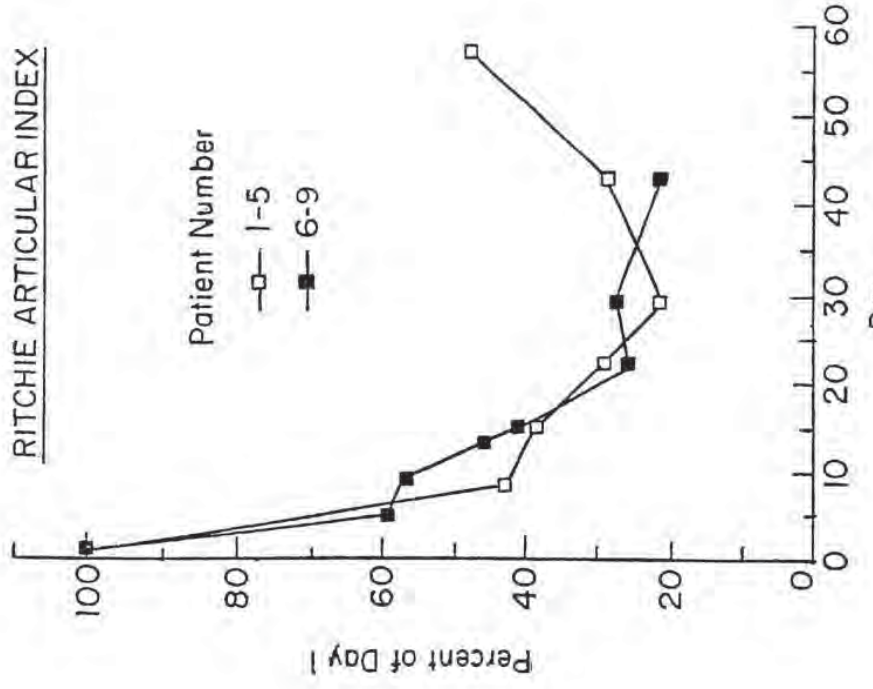

RITCHIE ARTICULAR INDEX
Patient Number
1-5
6-9
Percent of Day 1
Day
0
20
40
60
80
100
0
10
20
30
40
50
60

FIG. 19

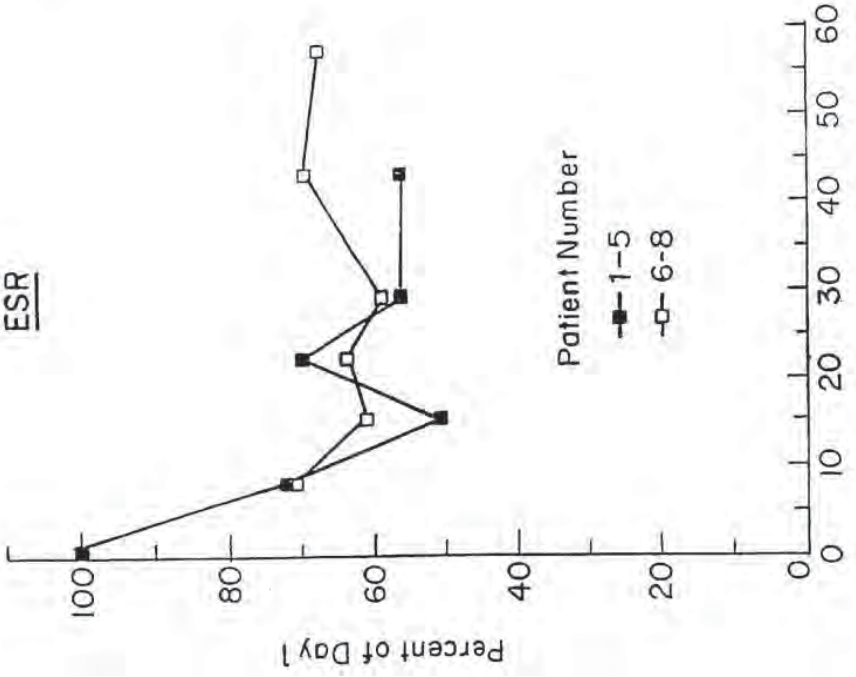

ESR
Patient Number
1-5
6-8
Percent of Day 1
Day
0
10
20
30
40
50
60
20
40
60
80
100

FIG. 22

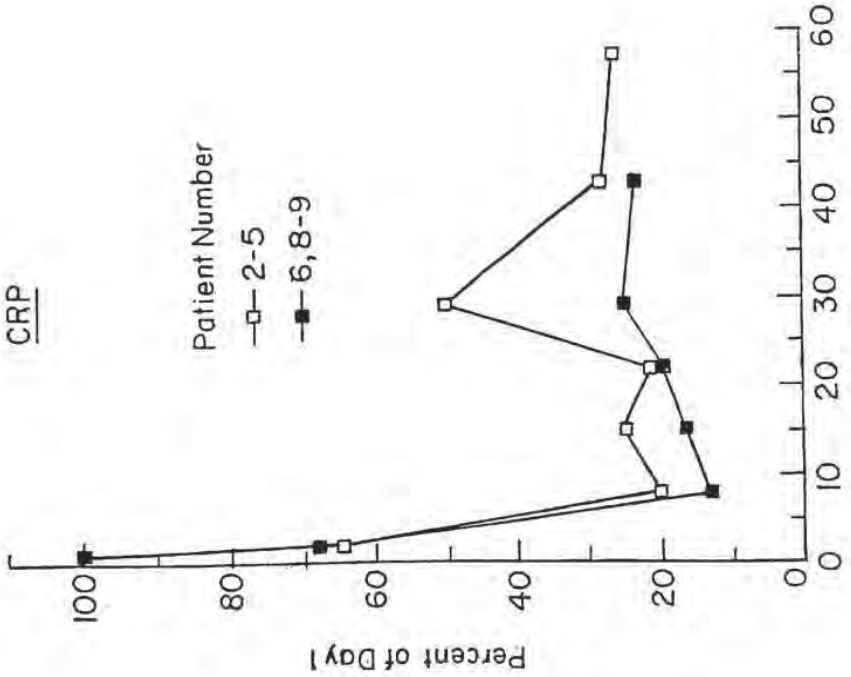

CRP
Patient Number
2-5
6,8-9
Percent of Day 1
Day
0
10
20
30
40
50
60
20
40
60
80
100

FIG. 21

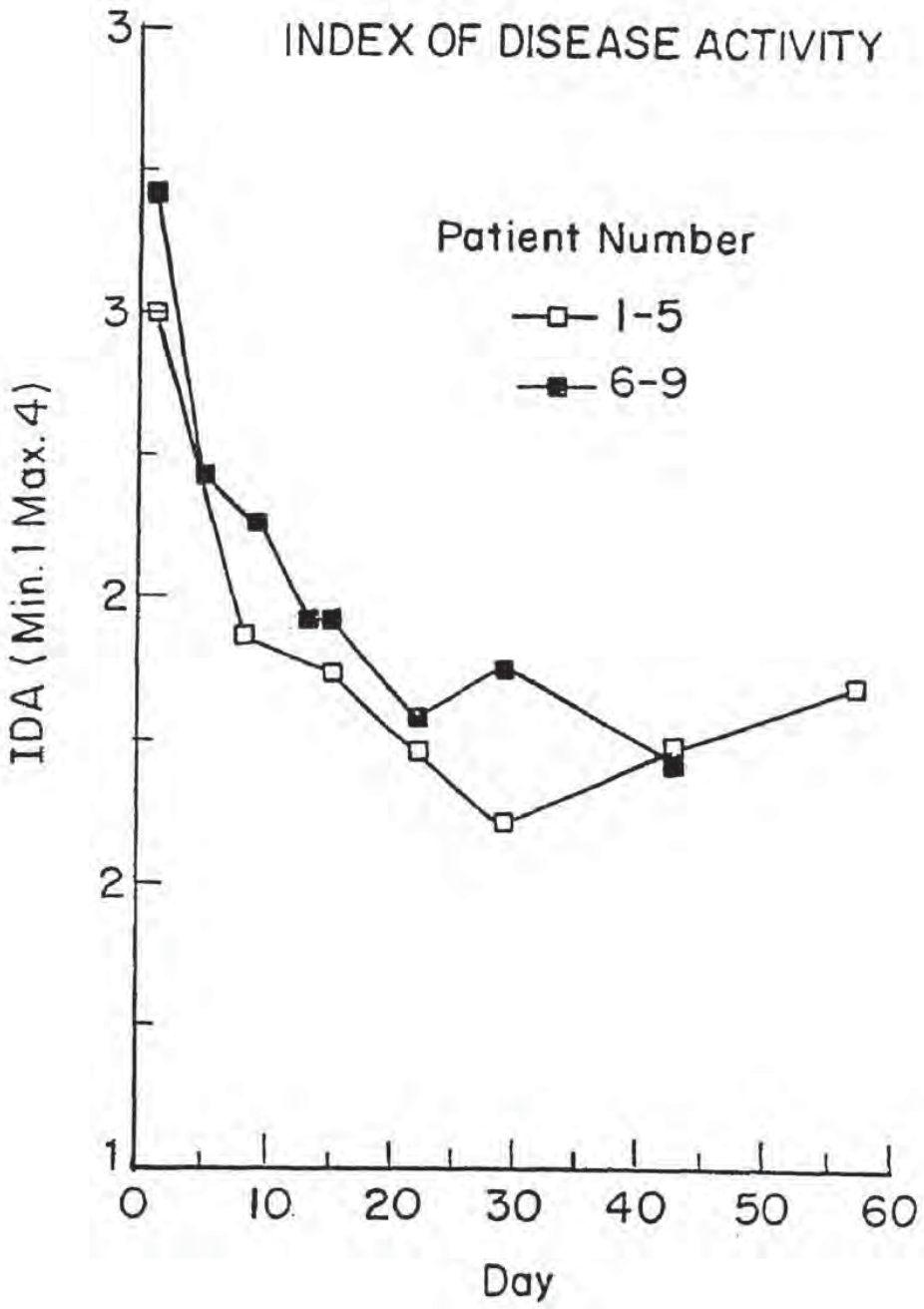

INDEX OF DISEASE ACTIVITY
Patient Number
1-5
6-9
IDA (Min. 1 Max. 4)
3
3
2
2
1
0
10
20
30
40
50
60
Day

FIG. 23

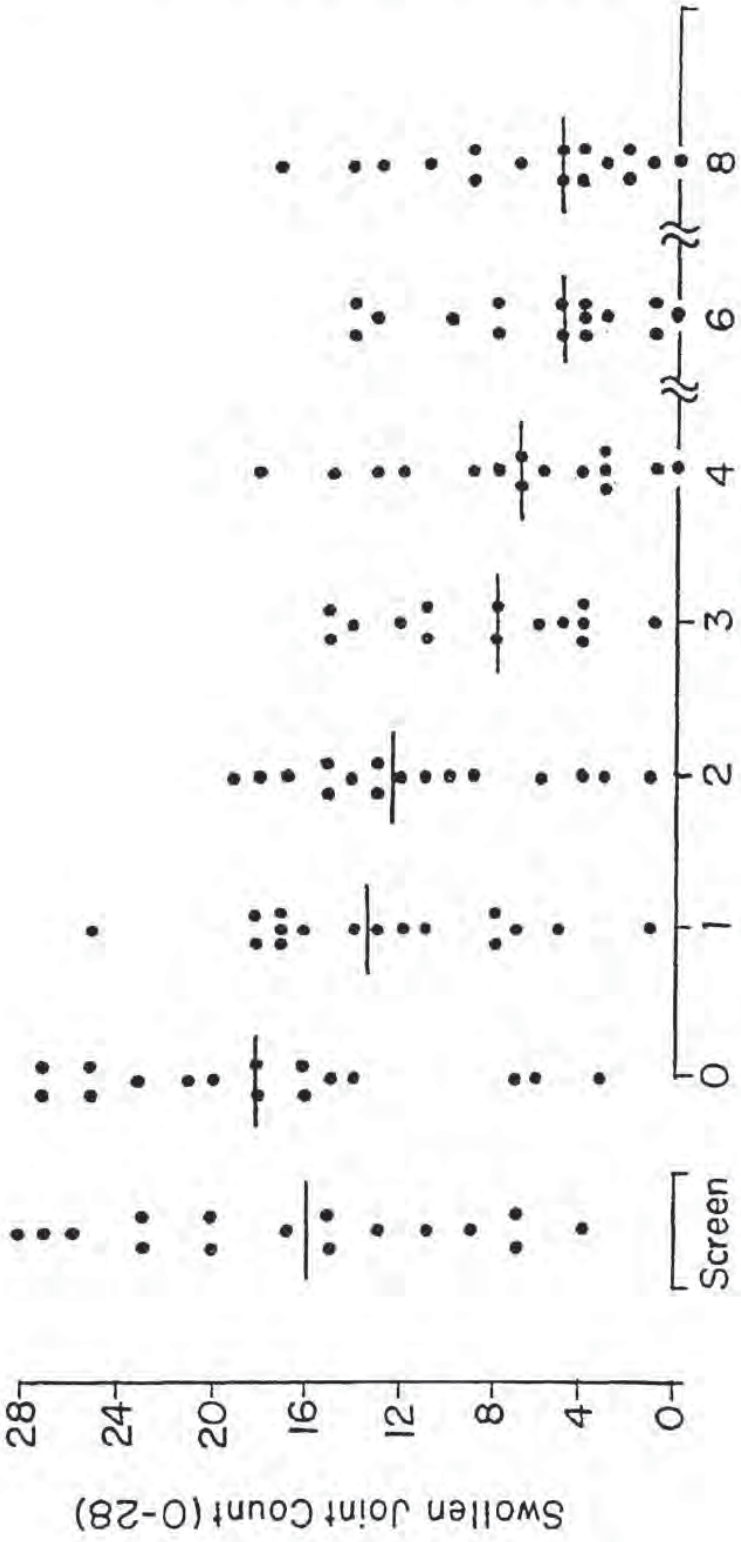

Swollen Joint Count (0-28)
28
24
20
16
12
8
4
0
Screen
0
1
2
3
4
6
8
Weeks

FIG. 24

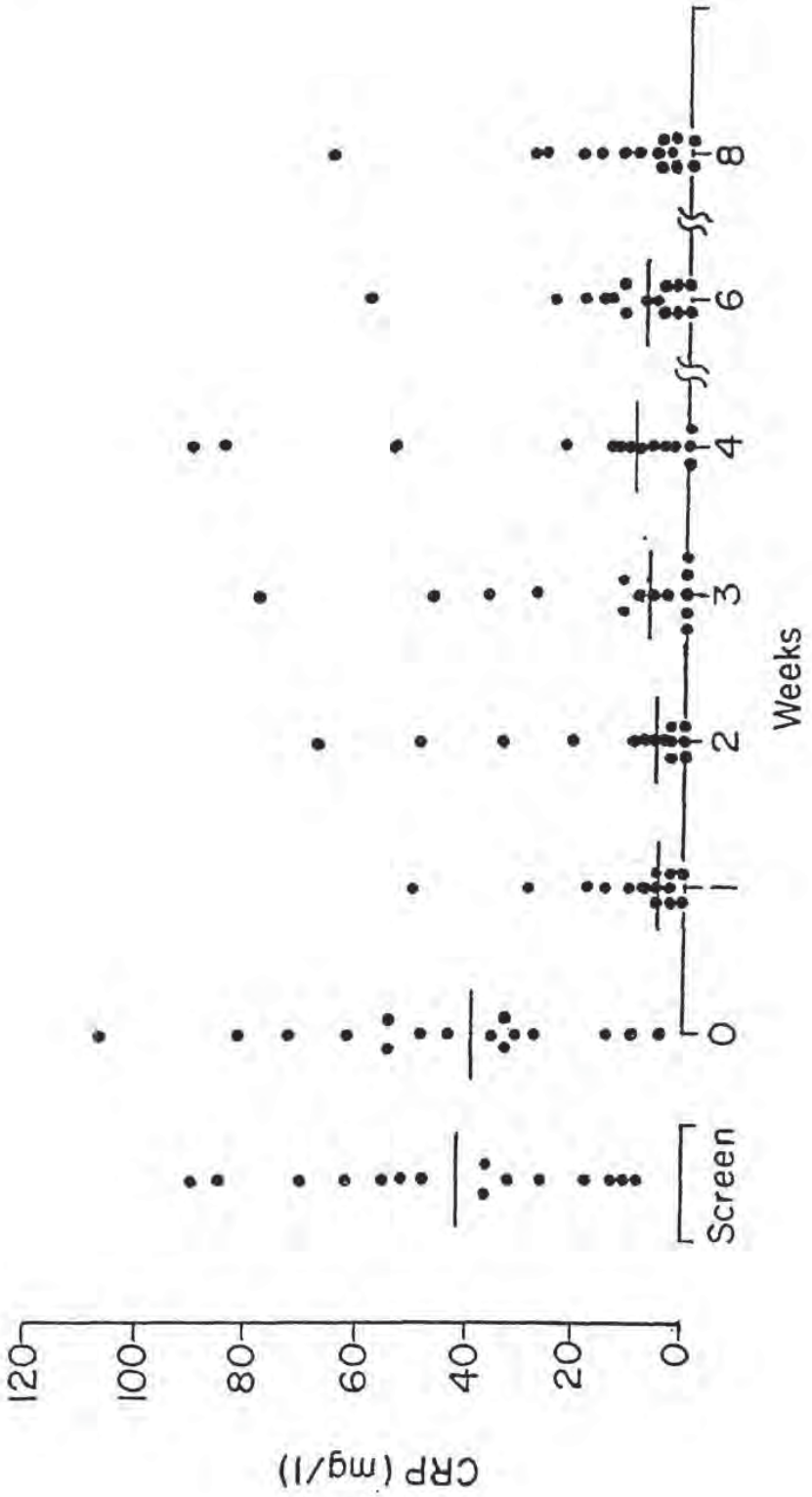


FIG. 25

FIG. 26A

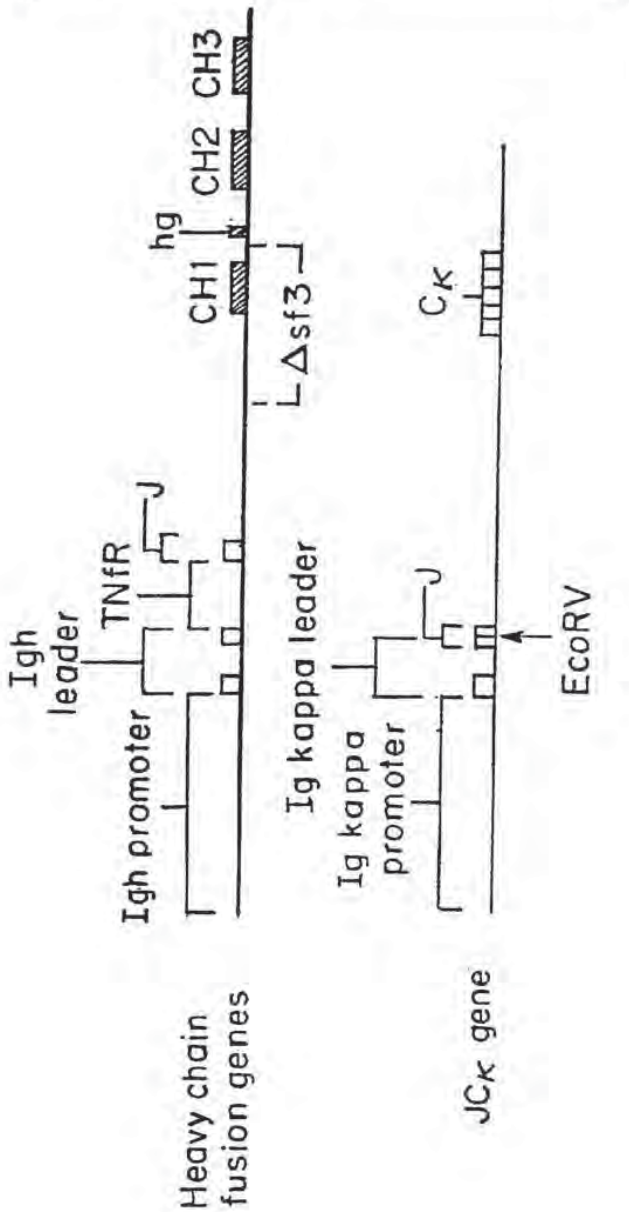

Igh leader
TNfR
J
Igh promoter
hg
CH1
CH2
CH3
Δsf3
Heavy chain fusion genes
Ig kappa leader
J
Ig kappa promoter
Cκ
EcoRV
JCκ gene

FIG. 26B

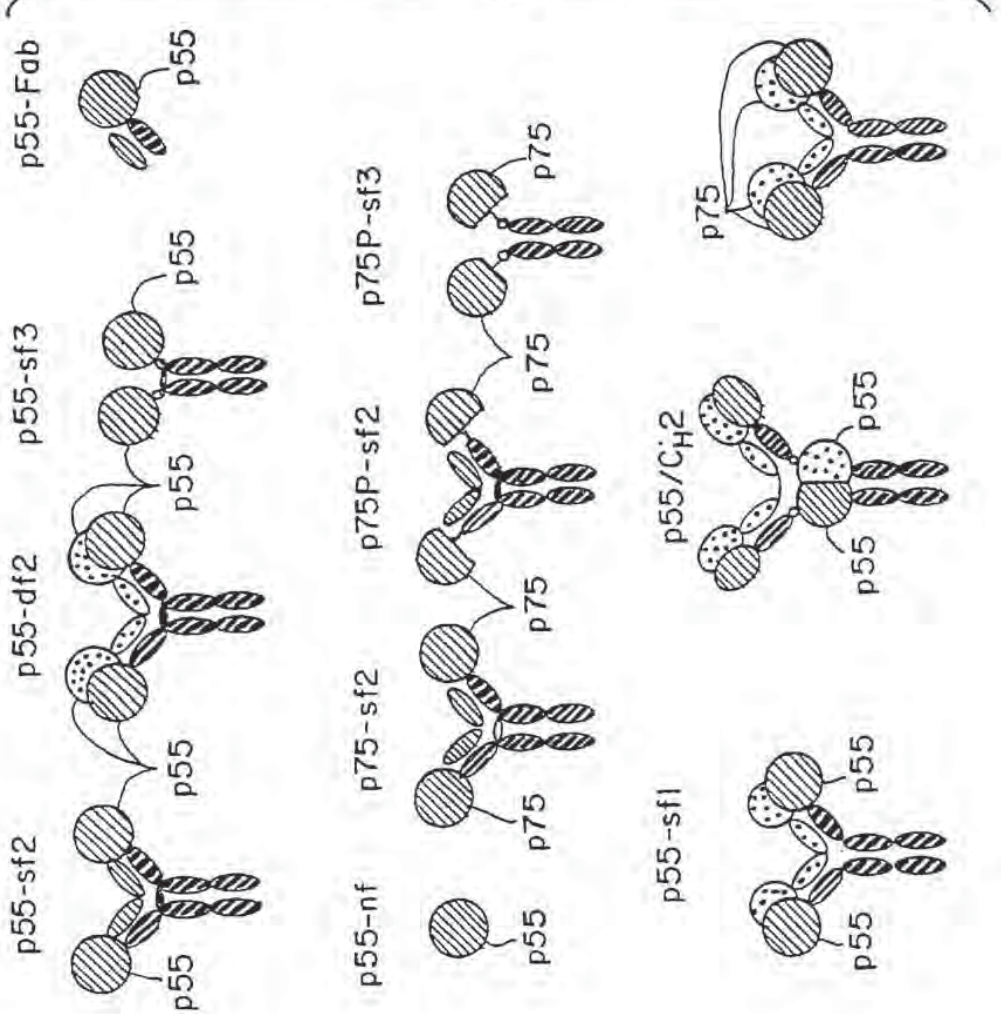
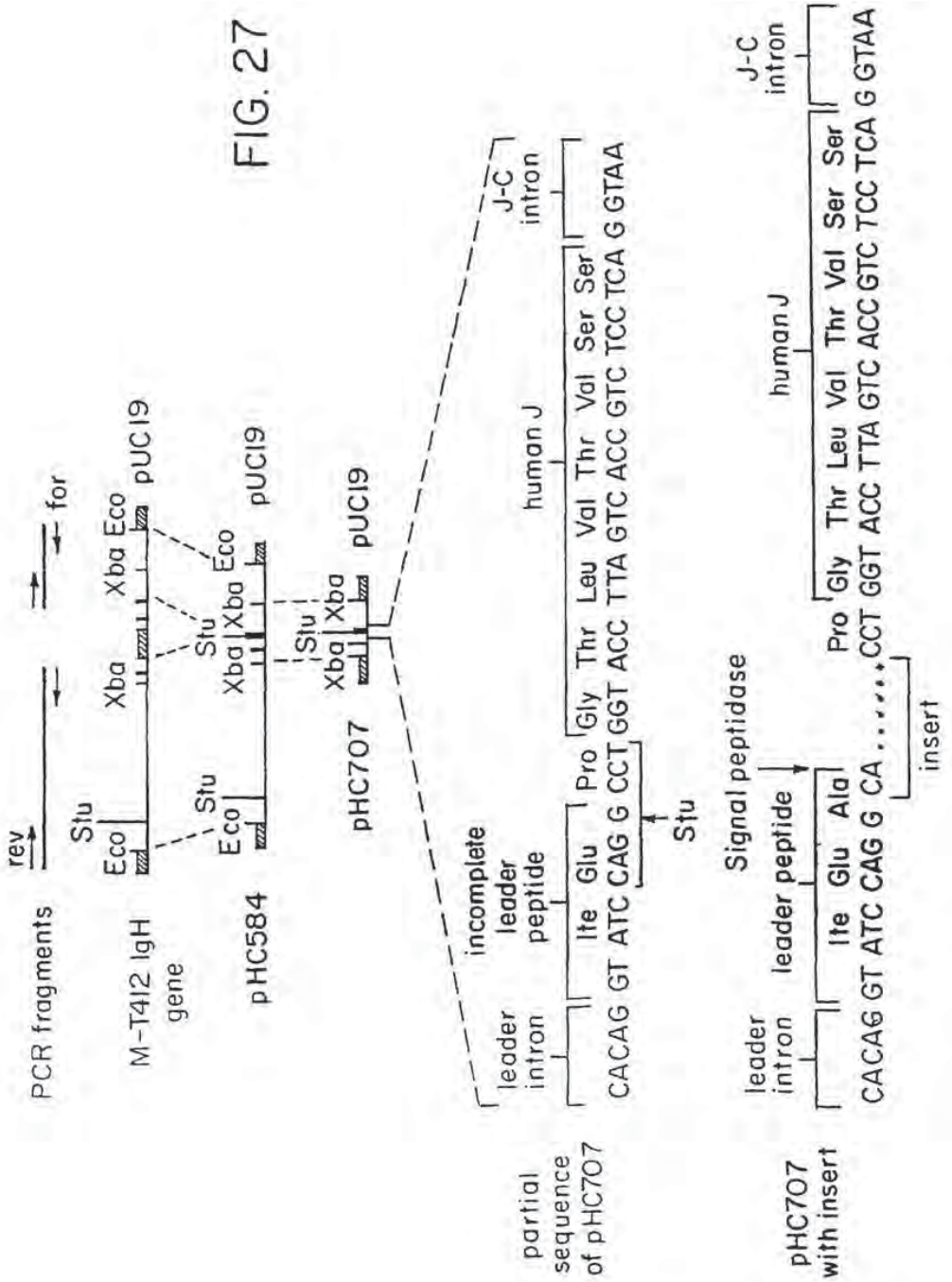

p55-Fab
p55
p55-sf3
p55
p55
p55-df2
p55
p55-sf2
p55
p75P-sf3
p75
p75
p75P-sf2
p75
p75
p75-sf2
p75
p55-nf
p55
p75
p55/C'H2
p55
p55
p55-sf1
p55
p55

FIG. 27


PCR fragments
rev
for
Stu
Eco
Xba
Xba Eco
pUC19
M-T412 IgH gene
pHC584
Stu
Eco
Xba
Xba
Eco
pUC19
Stu
Xba
Xba
pUC19
pHC707
incomplete leader peptide
leader intron
human J
J-C intron
partial sequence of pHC707
Ile Glu Pro Gly Thr Leu Val Thr Val Ser Ser
CACAG GT ATC CAG G CCT GGT ACC TTA GTC ACC GTC TCC TCA G GTAA
Stu
Signal peptidase
leader intron
leader peptide
human J
J-C intron
pHC707 with insert
Ile Glu Ala Pro Gly Thr Leu Val Thr Val Ser Ser
CACAG GT ATC CAG G CA . . . . . CCT GGT ACC TTA GTC ACC GTC TCC TCA G GTAA
insert

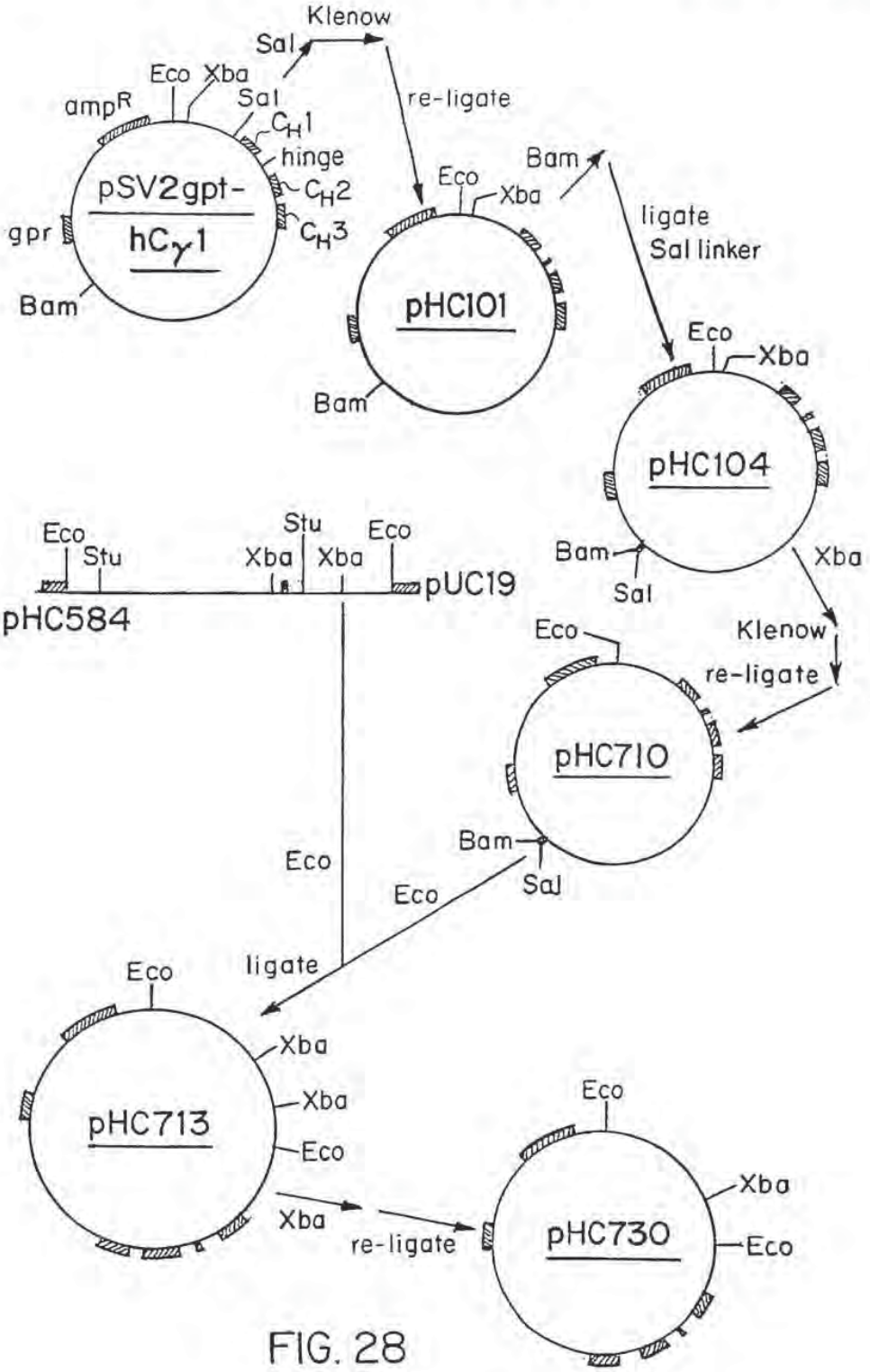

Klenow
Sal
Eco Xba
Sal
ampR
C_H1
hinge
C_H2
C_H3
pSV2gpt-
hCγ1
gpr
Bam
re-ligate
Eco
Xba
pHC101
Bam
Bam
ligate
Sal linker
Eco
Xba
pHC104
Bam
Sal
Xba
Eco
Stu
Xba
Xba
Eco
pUC19
pHC584
Klenow
re-ligate
Eco
pHC710
Bam
Sal
Eco
Eco
ligate
Eco
Xba
Xba
Eco
pHC713
Xba
re-ligate
Eco
Xba
pHC730
Eco

FIG. 28

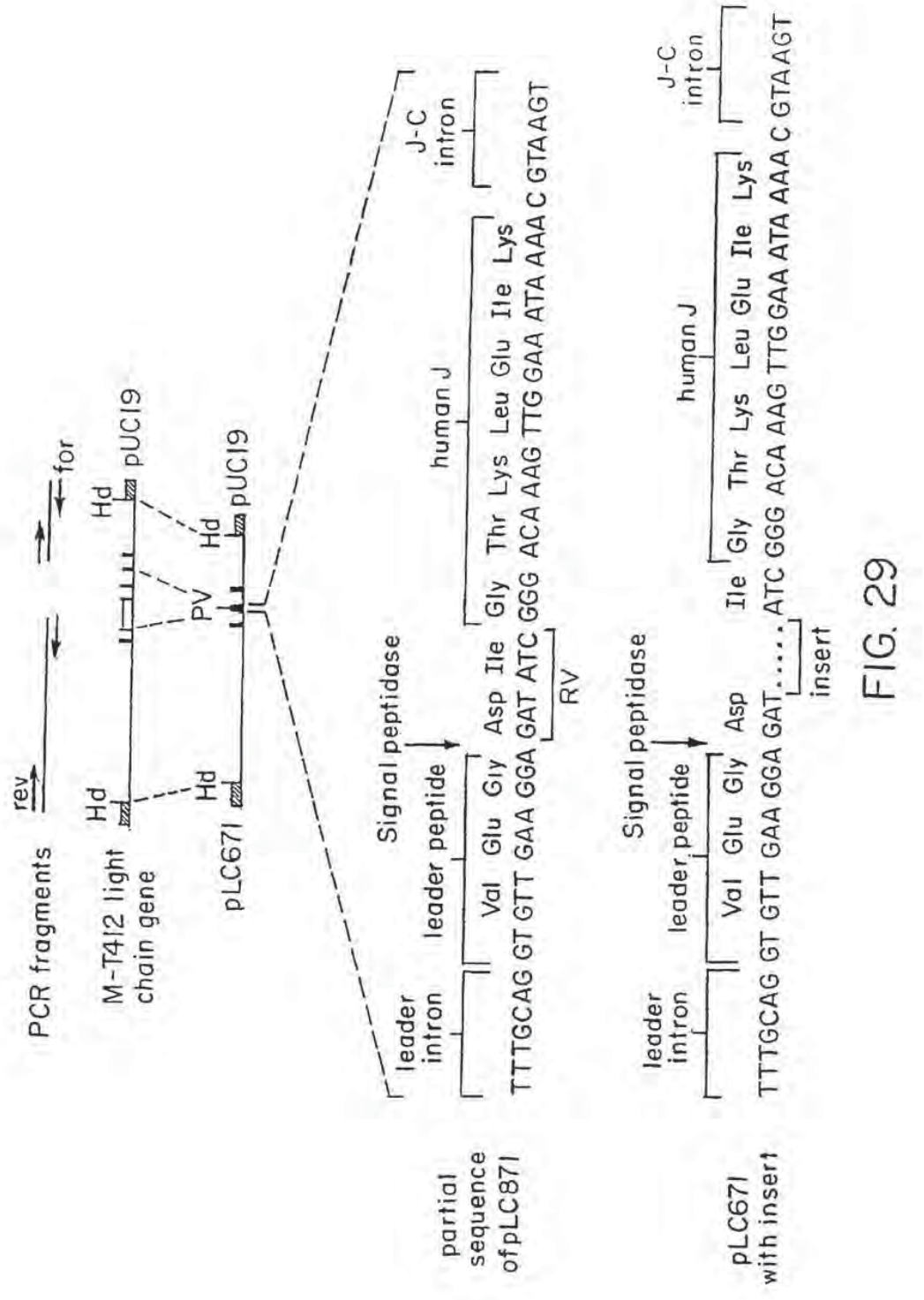

PCR fragments
rev
for
M-T412 light chain gene
Hd
Hd
pUC19
PV
Hd
pLC671
pUC19
partial sequence of pLC871
leader intron
Signal peptidase
leader peptide
Val Glu Gly Asp Ile Gly Thr Lys Leu Glu Ile Lys
TTTGCAG GT GTT GAA GGA GAT ATC GGG ACA AAG TTG GAA ATA AAA C GTAAGT
human J
J-C intron
RV
pLC671 with insert
leader intron
Signal peptidase
leader peptide
Val Glu Gly Asp Ile Gly Thr Lys Leu Glu Ile Lys
TTTGCAG GT GTT GAA GGA GAT.....ATC GGG ACA AAG TTG GAA ATA AAA C GTAAGT
insert
human J
J-C intron

FIG. 29

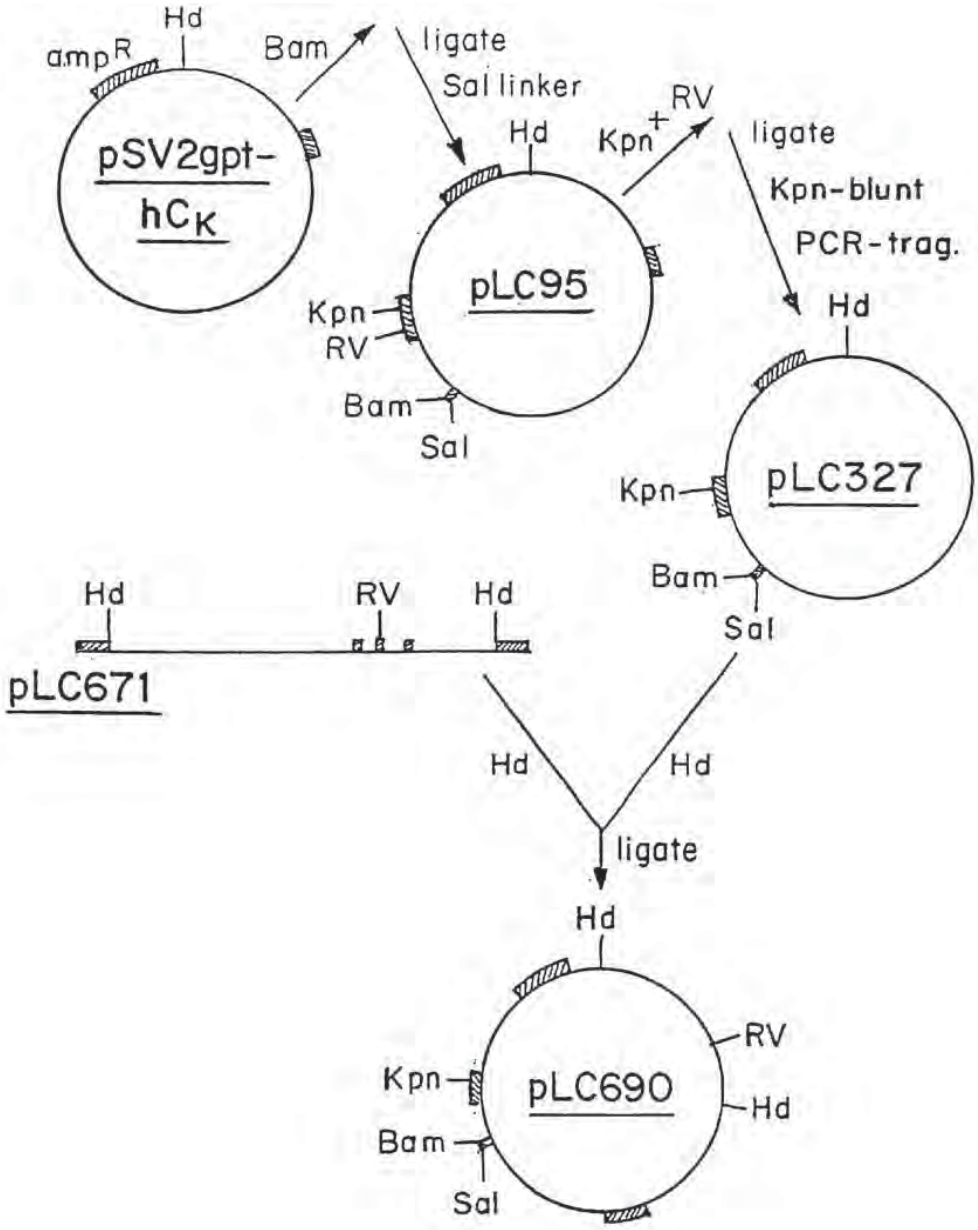

Hd
ampR
Bam
pSV2gpt-
hCK
ligate
Sal linker
Hd
Kpn+
RV
ligate
Kpn-blunt
PCR-trag.
pLC95
Kpn
RV
Bam
Sal
Hd
pLC327
Kpn
Bam
Sal
Hd
RV
Hd
pLC671
Hd
Hd
ligate
Hd
RV
Hd
pLC690
Kpn
Bam
Sal

FIG. 30

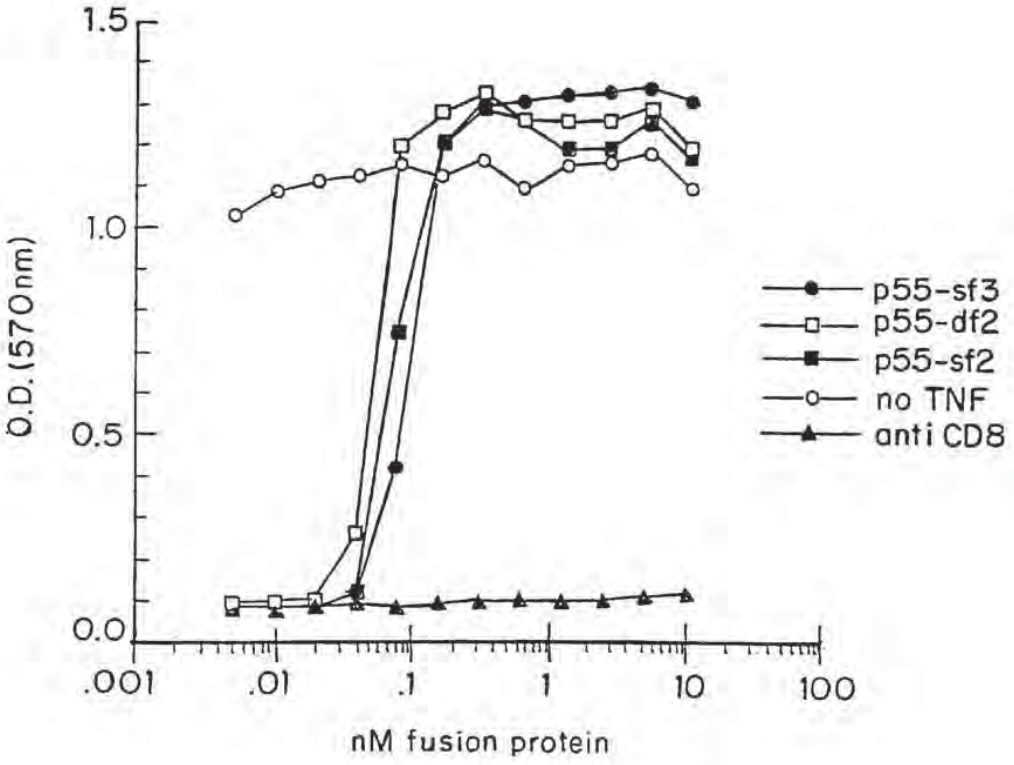
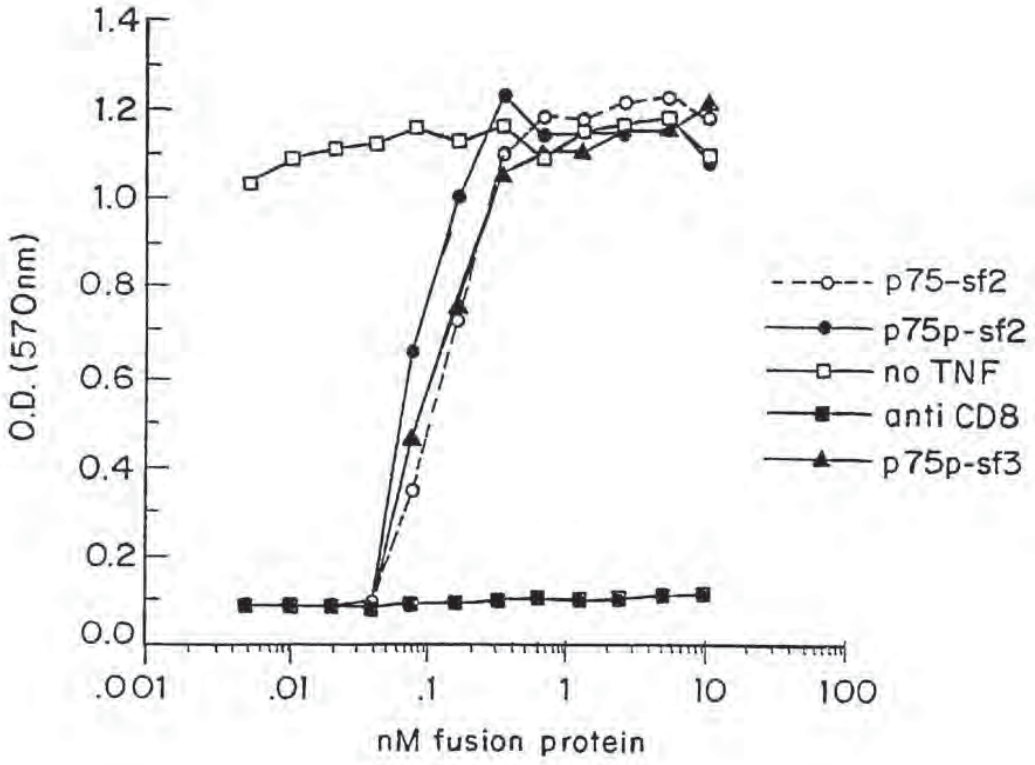

1.5
1.0
0.5
0.0
O.D. (570nm)
.001
.01
.1
1
10
100
nM fusion protein
p55-sf3
p55-df2
p55-sf2
no TNF
anti CD8

FIG. 31A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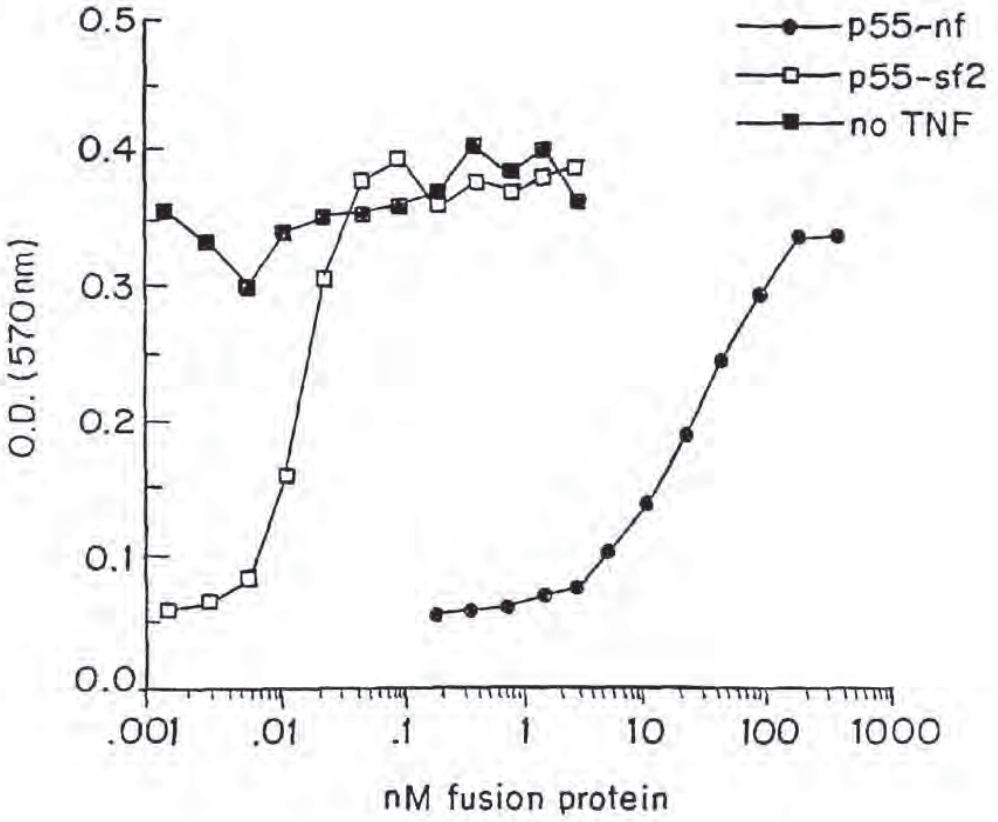
1.2
1.0
0.8
0.6
0.4
0.2
0.0
O.D. (570nm)
.001
.01
.1
1
10
100
nM fusion protein
p75-sf2
p75p-sf2
no TNF
anti CD8
p75p-sf3

FIG. 31B


0.5
0.4
0.3
0.2
0.1
0.0
O.D. (570 nm)
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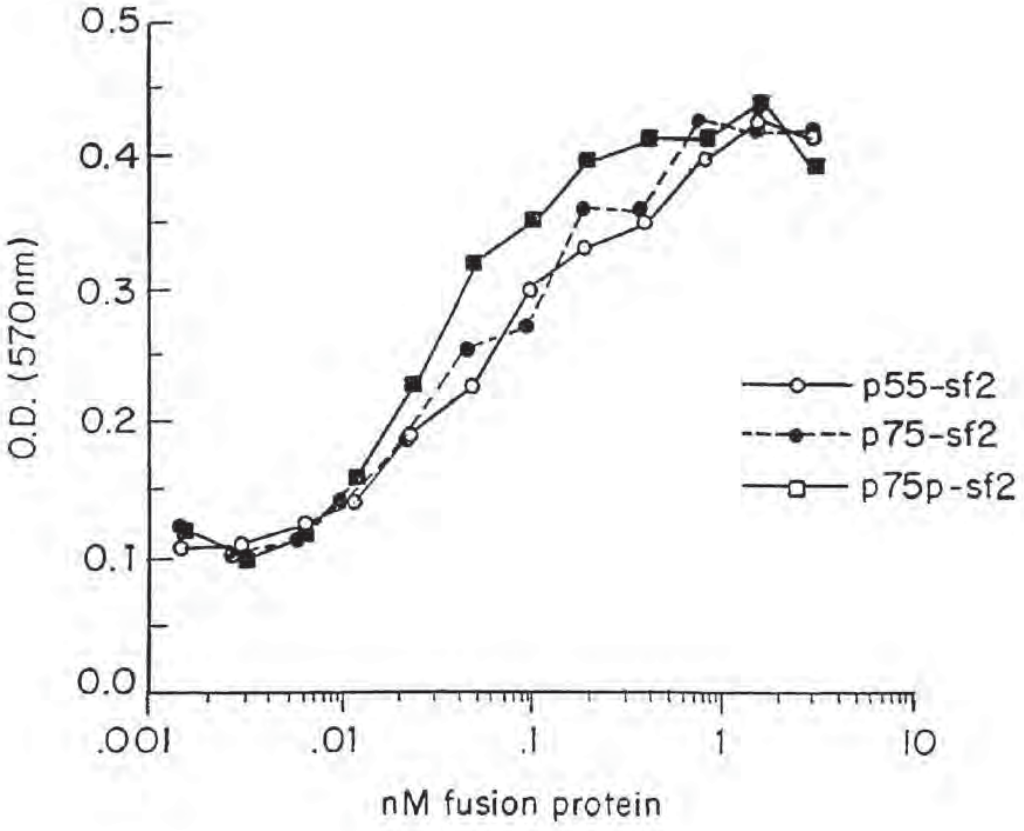
.01
.1
1
10
100
1000
nM fusion protein
p55-nf
p55-sf2
no TNF

FIG. 31C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0.4
0.3
0.2
0.1
0.0
O.D. (570nm)
.001
.01
.1
1
10
nM fusion protein
p55-sf2
p75-sf2
p75p-sf2

FIG. 32

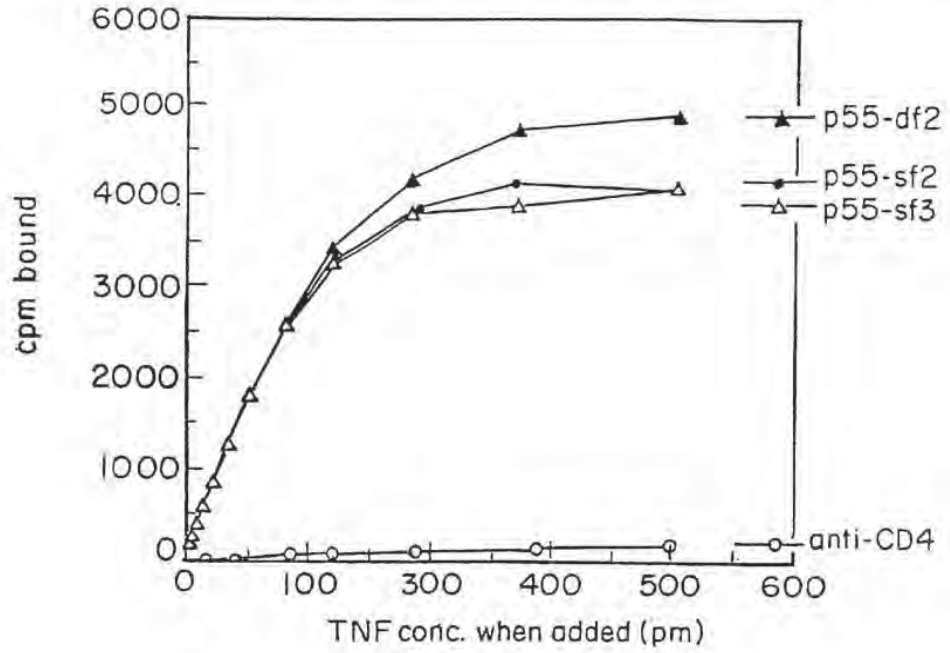

FIG. 33A

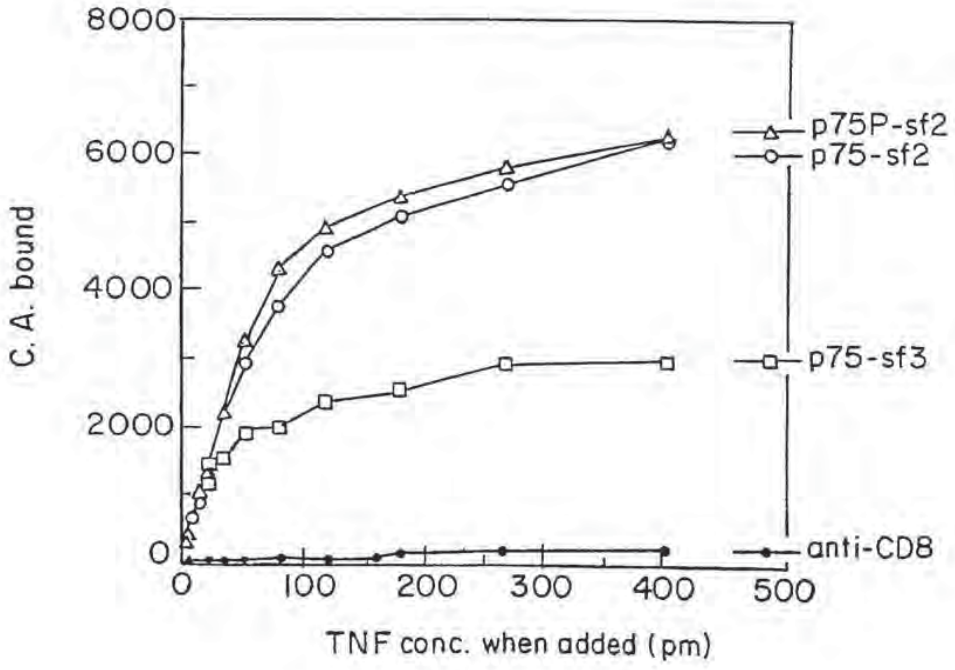

FIG. 33B

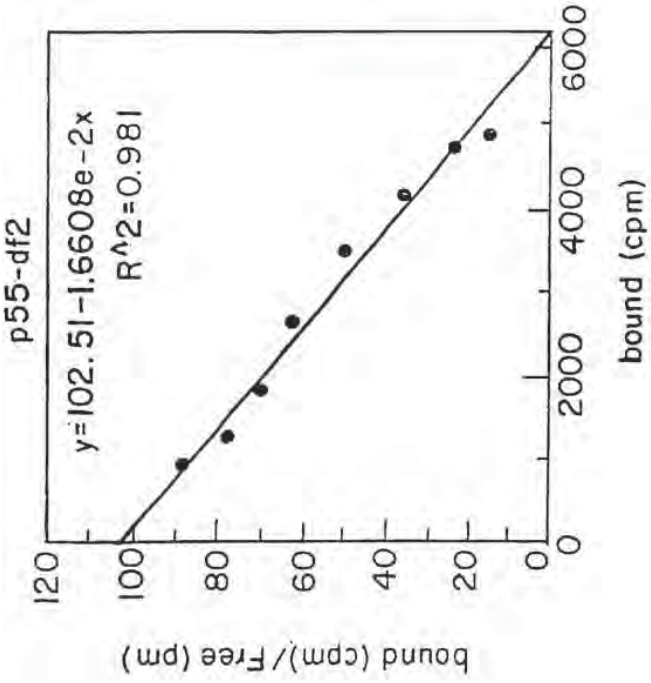

FIG. 33D

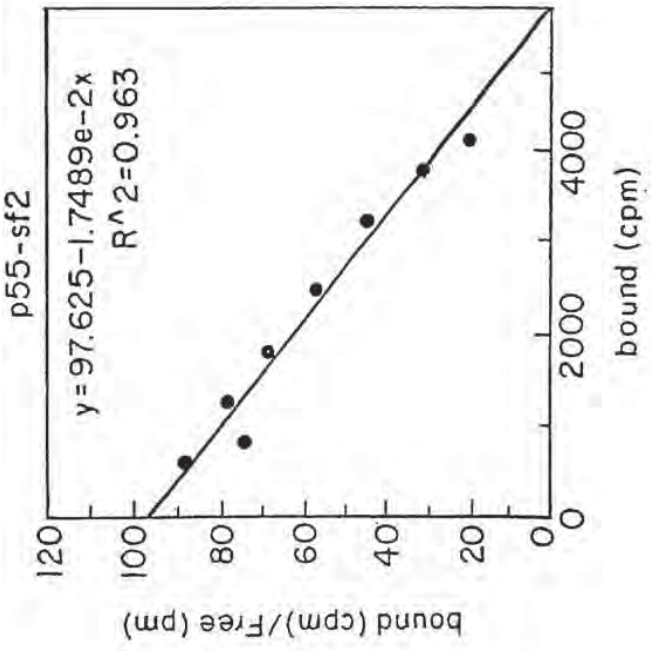

FIG. 33C

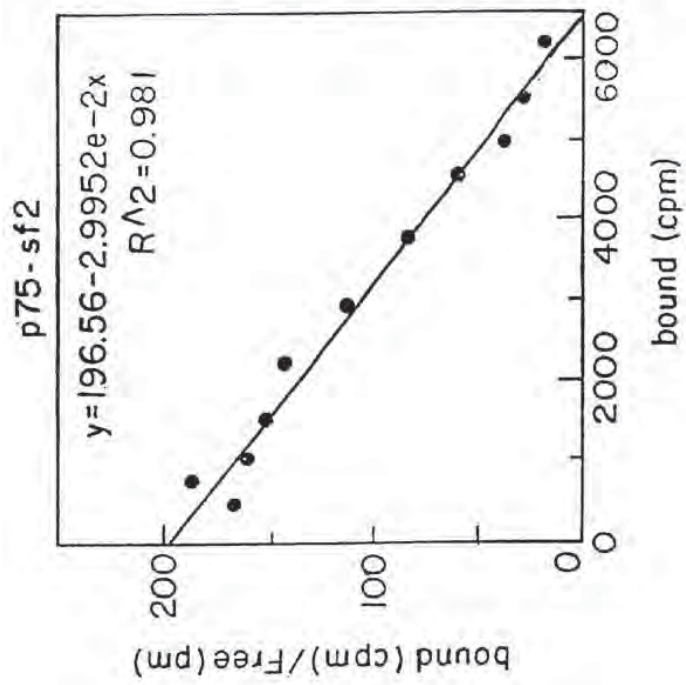

FIG. 33F

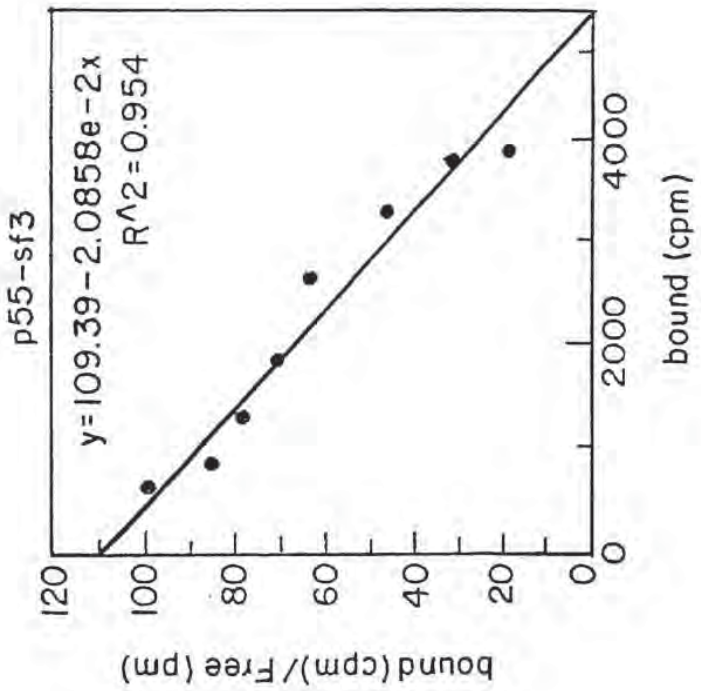

FIG. 33E

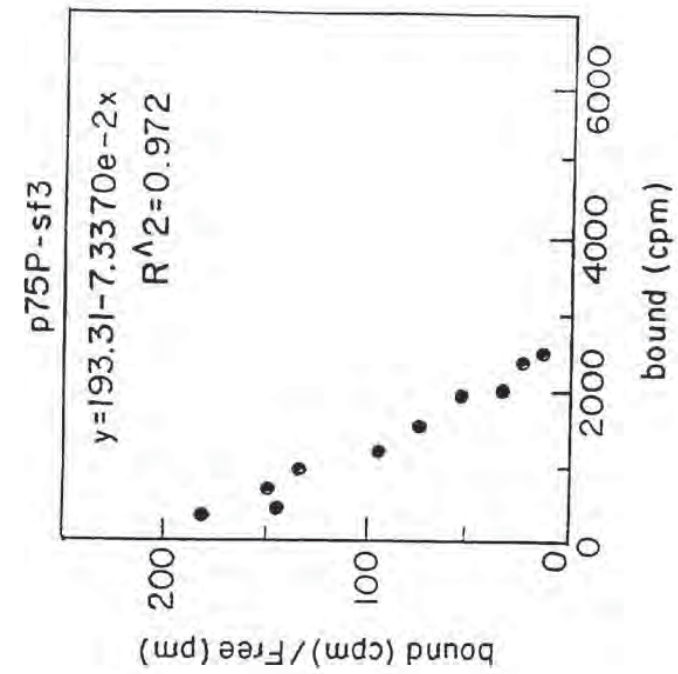

FIG. 33H

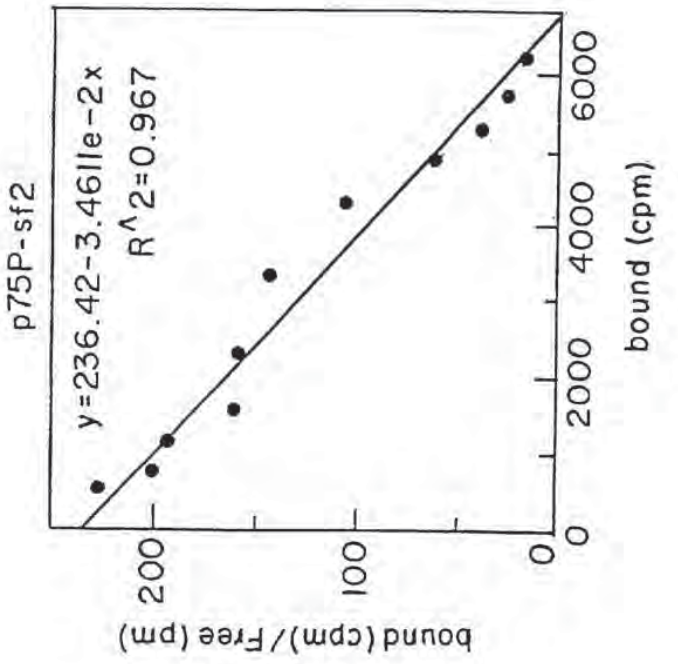

FIG. 33G

ANTI-TNFA ANTIBODIES AND ASSAYS EMPLOYING ANTI-TNFA ANTIBODIES

This application is a continuation-in-part of each of U.S. application Ser. No. 08/010,406, filed Jan. 29, 1993, now abandoned; and CIP of U.S. application Ser. No. 08/013,413, filed Feb. 2, 1993, now abandoned, which is a continuation-in-part of U.S. application Ser. No. 07/943,852, filed Sep. 11, 1992, now abandoned, which is a continuation-in-part of U.S. application Ser. No. 07/853,606, filed Mar. 18, 1992, now abandoned, which is a continuation-in-part of U.S. application Ser. No. 07/670,827, filed Mar. 18, 1991, now abandoned. Each of the above non-abandoned applications are entirely incorporated herein by reference.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention in the field of immunology and medicine relates to anti-human tumor necrosis factor-alpha (hTNFα) antibodies and peptides and nucleic acids encoding therefor, and to pharmaceutical and diagnostic compositions and production, diagnostic and therapeutic methods thereof, and to methods for treating TNF-mediated pathologies.

2. Description of the Background Art

Tumor Necrosis Factor: Monocytes and macrophages secrete cytokines known as tumor necrosis factor-α (TNFα) and tumor necrosis factor-β (TNFβ) in response to endotoxin or other stimuli. TNFα is a soluble homotrimer of 17 kD protein subunits (Smith, et al., *J. Biol. Chem.* 262:6951–6954 (1987)). A membrane-bound 26 kD precursor form of TNF also exists (Kriegler, et al., *Cell* 53:45–53 (1988)). For reviews of TNF, see Beutler, et al., *Nature* 320:584 (1986), *Old, Science* 230:630 (1986), and Le, et al., *Lab. Invest.* 56:234 (1987).

Cells other than monocytes or macrophages also make TNFα. For example, human non-monocytic tumor cell lines produce TNF (Rubin, et al., *J. Exp. Med.* 164:1350 (1986); Spriggs, et al., *Proc. Natl. Acad. Sci. USA* 84:6563 (1987)). $CD4^+$ and $CD8^+$ peripheral blood T lymphocytes and some cultured T and B cell lines (Cuturi, et al., *J. Exp. Med.* 165:1581 (1987); Sung, et al., *J. Exp. Med.* 168:1539 (1988)) also produce TNFα.

TNF causes pro-inflammatory actions which result in tissue injury, such as inducing procoagulant activity on vascular endothelial cells (Pober, et al., *J. Immunol.* 136:1680 (1986)), increasing the adherence of neutrophils and lymphocytes (Pober, et al., *J. Immunol.* 138:3319 (1987)), and stimulating the release of platelet activating factor from macrophages, neutrophils and vascular endothelial cells (Camussi, et al., *J. Exp. Med.* 166:1390 (1987)).

Recent evidence associates TNF with infections (Cerami, et al., *Immunol. Today* 9:28 (1988)), immune disorders, neoplastic pathologies (Oliff, et al., *Cell* 50:555 (1987)), autoimmune pathologies and graft-versus host pathologies (Piguet, et al., *J. Exp. Med.* 166:1280 (1987)). The association of TNF with cancer and infectious pathologies is often related to the host's catabolic state. Cancer patients suffer from weight loss, usually associated with anorexia.

The extensive wasting which is associated with cancer, and other diseases, is known as "cachexia" (Kern, et al. (*J. Parent. Enter. Nutr.* 12:286–298 (1988)). Cachexia includes progressive weight loss, anorexia, and persistent erosion of body mass in response to a malignant growth. The fundamental physiological derangement can relate to a decline in food intake relative to energy expenditure. The cachectic state causes most cancer morbidity and mortality. TNF can mediate cachexia in cancer, infectious pathology, and other catabolic states.

TNF also plays a central role in gram-negative sepsis and endotoxic shock (Michie, et al., *Br. J. Surg.* 76:670–671 (1989); Debets, et al., *Second Vienna Shock Forum*, p. 463–466 (1989); Simpson, et al., *Crit. Care Clin.* 5:27–47 (1989)), including fever, malaise, anorexia, and cachexia. Endotoxin strongly activates monocyte/macrophage production and secretion of TNF and other cytokines (Kornbluth, et al., *J. Immunol.* 137:2585–2591 (1986)). TNF and other monocyte-derived cytokines mediate the metabolic and neurohormonal responses to endotoxin (Michie, et al., *New. Engl. J. Med.* 318:1481–1486 (1988)). Endotoxin administration to human volunteers produces acute illness with flu-like symptoms including fever, tachycardia, increased metabolic rate and stress hormone release (Revhaug, et al., *Arch. Surg.* 123:162–170 (1988)). Circulating TNF increases in patients suffering from Gram-negative sepsis (Waage, et al., *Lancet* 1:355–357 (1987); Hammerle, et al., *Second Vienna Shock Forum* p. 715–718 (1989); Debets, et al., *Crit. Care Med.* 17:489–497 (1989); Calandra, et al., *J. Infect. Dis.* 161:982–987 (1990)).

TNF Antibodies

Polyclonal murine antibodies to TNF are disclosed by Cerami et al. (EPO Patent Publication 0212489, Mar. 4, 1987). Such antibodies were said to be useful in diagnostic immunoassays and in therapy of shock in bacterial infections.

Rubin et al. (EPO Patent Publication 0218868, Apr. 22, 1987) discloses murine monoclonal antibodies to human TNF, the hybridomas secreting such antibodies, methods of producing such murine antibodies, and the use of such murine antibodies in immunoassay of TNF.

Yone et al. (EPO Patent Publication 0288088, Oct. 26, 1988) discloses anti-TNF murine antibodies, including mAbs, and their utility in immunoassay diagnosis of pathologies, in particular Kawasaki's pathology and bacterial infection. The body fluids of patients with Kawasaki's pathology (infantile acute febrile mucocutaneous lymph node syndrome; Kawasaki, *Allergy* 16:178 (1967); Kawasaki, *Shonica* (*Pediatrics*) 26:935 (1985)) were said to contain elevated TNF levels which were related to progress of the pathology (Yone et al., infra).

Other investigators have described rodent or murine mAbs specific for recombinant human TNF which had neutralizing activity in vitro (Liang, et al. (*Biochem. Biophys. Res. Comm.* 137:847–854 (1986); Meager, et al., *Hybridoma* 6:305–311 (1987); Fendly et al., *Hybridoma* 6:359–369 (1987); Bringman, et al., *Hybridoma* 6:489–507 (1987); Hirai, et al., *J. Immunol. Meth.* 96:57–62 (1987); Moller, et al. (*Cytokine* 2:162–169 (1990)). Some of these mAbs were used to map epitopes of human TNF and develop enzyme immunoassays (Fendly et al., infra; Hirai et al., infra; Moller et al., infra) and to assist in the purification of recombinant TNF (Bringman et al., infra). However, these studies do not provide a basis for producing TNF neutralizing antibodies that can be used for in vivo diagnostic or therapeutic uses in humans, due to immunogenicity, lack of specificity and/or pharmaceutical suitability.

Neutralizing antisera or mAbs to TNF have been shown in mammals other than man to abrogate adverse physiological changes and prevent death after lethal challenge in experimental endotoxemia and bacteremia. This effect has been demonstrated, e.g., in rodent lethality assays and in primate pathology model systems (Mathison, et al., *J. Clin.*

Invest. 81:1925–1937 (1988); Beutler, et al., *Science* 229:869–871 (1985); Tracey, et al., *Nature* 330:662–664 (1987); Shimamoto, et al., *Immunol. Lett.* 17:311–318 (1988); Silva, et al., *J. Infect. Dis.* 162:421–427 (1990); Opal, et al., *J. Infect. Dis.* 161:1148–1152 (1990); Hinshaw, et al., *Circ. Shock* 30:279–292 (1990)).

Putative receptor binding loci of hTNF has been disclosed by Eck and Sprang (*J. Biol. Chem.* 264(29), 17595–17605 (1989), who identified the receptor binding loci of TNF-α as consisting of amino acids 11–13, 37–42, 49–57 and 155–157.

PCT publication WO91/02078 (1991) discloses TNF ligands which can bind to monoclonal antibodies having the following epitopes: at least one of 1–20, 56–77, and 108–127; at least two of 1–20, 56–77, 108–127 and 138–149; all of 1–18, 58–65, 115–125 and 138–149; all of 1–18, and 108–128; all of 56–79, 110–127 and 135– or 136–155; all of 1–30, 117–128 and 141–153; all of 1–26, 117–128 and 141–153; all of 22–40, 49–96 or 49–97, 110–127 and 136–153; all of 12–22, 36–45, 96–105 and 132–157; both of 1–20 and 76–90; all of 22–40, 69–97, 105–128 and 135–155; all of 22–31 and 146–157; all of 22–40 and 49–98; at least one of 22–40, 49–98 and 69–97, both of 22–40 and 70–87.

To date, experience with anti-TNF murine mAb therapy in humans has been limited. In a phase I study, fourteen patients with severe septic shock were administered a murine anti-TNF mAb in a single dose from 0.4–10 mg/kg (Exley, A. R. et al., *Lancet* 335:1275–1277 (1990)). However, seven of the fourteen patients developed a human anti-murine antibody response to the treatment, which treatment suffers from the known problems due to immuriogenicity from the use of murine heavy and light chain portions of the antibody. Such immunogenicity causes decreased effectiveness of continued administration and can render treatment ineffective, in patients undergoing diagnostic or therapeutic administration of murine anti-TNF antibodies.

Administration of murine TNF mAb to patients suffering from severe graft versus host pathology has also been reported (Herve, et al., *Lymphoma Res.* 9:591 (1990)).

TNF Receptors

The numerous biological effects of TNFα and the closely related cytokine, TNFβ (lymphotoxin), are mediated by two TNF transmembrane receptors, both of which have been cloned. The p55 receptor (also termed TNF-R55, TNF-RI, or TNFRβ) is a 55 kd glycoprotein shown to transduce signals resulting in cytotoxic, anti-viral, and proliferative activities of TNFα.

The p75 receptor (also termed TNF-R75, TNF-RII, or TNFRα) is a 75 kDa glycoprotein that has also been shown to transduce cytotoxic and proliferative signals as well as signals resulting in the secretion of GM-CSF. The extracellular domains of the two receptors have 28% homology and have in common a set of four subdomains defined by numerous conserved cysteine residues. The p75 receptor differs, however, by having a region adjacent to the transmembrane domain that is rich in proline residues and contains sites for O-linked glycosylation. Interestingly, the cytoplasmic domains of the two receptors share no apparent homology which is consistent with observations that they can transduce different signals to the interior of the cell.

TNFα inhibiting proteins have been detected in normal human urine and in serum of patients with cancer or endotoxemia. These have since been shown to be the extracellular domains of TNF receptors derived by proteolytic cleavage of the transmembrane forms. Many of the same stimuli that result in TNFα release also result in the release of the soluble receptors, suggesting that these soluble TNFα inhibitors can serve as part of a negative feedback mechanism to control TNFα activity.

Aderka, et al., *Isrl. J. Med. Sci.* 28:126–130 (1992) discloses soluble forms of TNF receptors (sTNF-Rs) which specifically bind TNF and thus can compete with cell surface TNF receptors to bind TNF (Seckinger, et al., *J. Exp. Med.* 167:1511–1516 (1988); Engelmann, et al., *J. Biol. Chem.* 264:11974–11980 (1989)).

Loetscher, et al., *Cell* 61:351–359 (Apr. 20, 1990) discloses the cloning and expression of human 55 kd TNF receptor with the partial amino acid sequence, complete cDNA sequence and predicted amino acid sequence.

Schall et al., *Cell* 61:361–370 (Apr. 20, 1990), discloses molecular cloning and expression of a receptor for human TNF wherein an isolated cDNA clone including a receptor as a 415 amino acid protein with an apparent molecular weight of 28 kDa, as well as the cDNA sequence and predicted amino acid sequence.

Nophar, et al., *EMBO J.* 9(10):3269–3278 (1990) discloses soluble forms of TNF receptor and that the cDNA for type I TNF-R encodes both the cell surface and soluble forms of the receptor. The cDNA and predicted amino acid sequences are disclosed.

Engelmann, et al., *J. Biol. Chem.* 265(3):1531–1536 (1990), discloses TNF-binding proteins, purified from human urine, both having an approximate molecular weight of 30 kDa and binding TNF-α more effectively than TNF-β. Sequence data is not disclosed. See also Engelmann, et al., *J. Biol. Chem.* 264(20):11974–11980 (1989).

European Patent publication number 0 433 900 A1, published Jun. 26, 1991, owned by YEDA Research and Develoepemtn Co., Ltd., Wallach, et al., discloses TNF binding protein I (TBP-I), derivatives and analogs thereof, produced expression of a DNA encoding the entire human type I TNF receptor, or a soluble domain thereof.

PCT publication number WO 92/13095, published Aug. 6, 1992, owned by Synergen, Carmichael et al., discloses methods for treating tumor necrosis factor mediated diseases by administration of a therapeutically effective amount of a TNF inhibitor selected from a 30 kDa TNF inhibitor and a 40 kDa TNF inhibitor selected from the full length 40 kDa TNF inhibitor or modifications thereof.

European Patent Publication number 0 526 905 A2, published Oct. 2, 1993, owned by YEDA Research one Development Company, Ltd., Wallach et al., discloses multimers of the soluble forms of TNF receptors produced by either chemical or recombinant methods which are useful for protecting mammals from the diliterious effects of TNF, which include portions of the hp55 TNF-receptor.

PCT publication WO 92/07076, published Apr. 30, 1992, owned by Charring Cross Sunley Research Center, Feldman et al., discloses modified human TNFα receptor which consists of the first three cysteine-rich subdomain but lacks the fourth Cysteine-rich subdomain of the extracellular binding domain of the 55 kDa or 75 kDa TNF receptor for human TNF α, or an amino acid sequence having a homology of 90% or more with the TNF receptor sequences.

European Patent Publication 0 412 486 A1, published Feb. 13, 1991, owned by YEDA Research and Development Co., Ltd., Wallach et al., discloses antibodies to TNF binding protein I (TBP-I), and fragments thereof, which can be used as diagnostic assays or pharmaceutical agents, either inhibiting or mimicking the effects of TNF on cells.

European Patent Publication number 0 398 327 A1, published Nov. 22, 1990, owned by YEDA Research and Development Co., Ltd., Wallach et al., discloses TNF bind-

ing protein (TBP) isolated and purified having inhibitory activity on the cytotoxic effect of TNF, as well as TNF binding protein II and salts, functional derivatives precursors and active fractions thereof, as well as polyclonal and monoclonal antibodies to TNF binding protein II.

European Patent Publication 0 308 378 A2, published Mar. 22, 1989, owned by YEDA Research and Development Co., Ltd., Wallach, et al., discloses TNF inhibitory protein isolated and substantially purified, having activity to inhibit the binding of TNF to TNF receptors and to inhibit the cytotoxicity of TNF. Additionally disclosed are TNF inhibitory protein, salts, functional derivatives and active fractions thereof, used to antagonize the diliterious effects of TNF.

Accordingly, there is a need to provide novel TNF antibodies or peptides which overcome the problems of murine antibody immunogenicity and which provide reduced immunogenicity and increased neutralization activity.

Citation of documents herein is not intended as an admission that any of the documents cited herein is pertinent prior art, or an admission that the cited documents is considered material to the patentability of any of the claims of the present application. All statements as to the date or representation as to the contents of these documents is based on the information available to the applicant and does not constitute any admission as to the correctness of the dates or contents of these documents.

SUMMARY OF THE INVENTION

It is object of the present invention to overcome one or more deficiencies of the background art.

It is also an object of the present invention to provide methods having utility for in vitro, in situ and/or in vivo diagnosis and/or treatment of animal cells, tissues or pathologies associated with the presence of tumor necrosis factor (TNF), using anti-TNF antibodies and/or anti-TNF peptides.

Anti-TNF antibodies (Abs) are intended to include at least one of monoclonal rodent-human chimeric antibodies, rodent antibodies, human antibodies or any portions thereof, having at least one antigen binding region of an immunoglobulin variable region, which antibody binds TNF.

Anti-TNF peptides are capable of binding TNF under physiological conditions, and can include, but are not limited to, portions of a TNF receptor and/or portions or structural analogs of anti-TNF antibody antigen binding regions or variable regions. Such antibodies or peptides bind TNF with neutralizing and/or inhibiting biological activity.

Anti-TNF antibodies and/or anti-TNF peptides of the present invention can be routinely made and/or used according to methods of the present invention, such as, but not limited to synthetic methods, hybridomas, and/or recombinant cells expressing nucleic acid encoding such anti-TNF antibodies or peptides.

The present invention also provides antigenic polypeptides of hTNF, corresponding to peptides containing neutralizing epitopes or portions of TNF that, when such epitopes on TNF are bound by anti-TNF antibodies or peptides, neutralize or inhibit the biological activity of TNF in vitro, in situ or in vivo.

The present invention also provides anti-TNF antibodies and peptides in the form of pharmaceutical and/or diagnostic compounds and/or compositions, useful for the diagnostic and/or therapeutic methods of the present invention for diagnosing and/or treating TNF-related pathologies.

Anti-TNF Abs or anti-TNF peptides of the present invention are provided for use in diagnostic methods for detecting TNF in patients or animals suspected of suffering from conditions associated with abnormal TNF production, including methods wherein high affinity anti-TNF antibodies or peptides are contacted with a biological sample from a patient and an antigen-antibody reaction detected. Also included in the present invention are kits for detecting TNF in a solution using anti-TNF antibodies or peptides, preferably in detectably labeled form.

The present invention is also directed to an anti-hTNF chimeric antibody comprising two light chains and two heavy chains, each of the chains comprising at least part of a human constant region and at least part of a variable (V) region of non-human origin having specificity to human TNF, said antibody binding with high affinity to a inhibiting and/or neutralizing epitope of human TNF, such as the antibody cA2. The invention is also includes a fragments or a derivative such an antibody, such as one or more portions of the antibody chain, such as the heavy chain constant, joining, diversity or variable regions, or the light chain constant, joining or variable regions.

Methods are also provided for making and using anti-TNF antibodies and peptides for various utilities of the present invention, such as but not limited to, hybridoma, recombinant or chemical synthetic methods for producing anti-TNF antibodies or anti-TNF peptides according to the present invention; detecting TNF in a solution or cell; removing TNF from a solution or cell, inhibiting one or more biological activities of TNF in vitro, in situ or in vitro. Such removal can include treatment methods of the present invention for alleviating symptoms or pathologies involving TNF, such as, by not limited to bacterial, viral or parasitic infections, chronic inflammatory diseases, autoimmune diseases, malignancies, and/or neurodegenerative diseases.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a graph showing dose dependent binding of mouse mAb A2 to human TNFα.

FIG. **2** is a graph showing lack of recognition of heat-inactivated human TNFα by mAb A2.

FIG. **3** is a graph showing neutralization of in vitro TNF cytotoxicity by murine A2. Control: murine IgG1 anti-lipid A mAb (8A1) with natural human TNF. Average absorbance values for controls were as follows: no TNF added=1.08; natural TNF, no antibody=0.290; and recombinant TNF, no antibody=0.500.

FIG. **4** is a graph showing that mAb A2 and chimeric A2 do not inhibit or neutralize human lymphotoxin (TNFβ).

FIG. **5** is a graph showing that mAbs murine A2 and chimeric CA2 do not inhibit or neutralize murine TNFα.

FIGS. **6** and FIG. **7** are graphs showing that mAb A2 inhibits or neutralizes TNF produced by chimpanzee monocytes and rhTNFα.

FIGS. **8**A and **8**B provides schematic diagrams of the plasmids used for expression of the chimeric H (pA2HG1apgpt) and L (pA2HuKapgpt) chains of the chimeric A2 antibody.

FIGS. **9**A and **9**B is a graph showing results of a cross-blocking epitope ELISA with murine A2 (mA2) and chimeric (cA2) antibody competitors.

FIGS. **10**A and **10**B is a graph of a Scatchard analysis of ^{125}I-labelled mAb A2 (mA2) and chimeric A2 (cA2) binding to recombinant human TNFα immobilized on a microtiter plate. Each Ka value was calculated from the average of two independent determinations.

FIG. **11** is a graph showing neutralization of TNF cytotoxicity by chimeric A2. The control is a chimeric mouse/

human IgG1 anti-platelet mAb (7E3) reacting with natural human TNF. Average absorbance values for controls were: no TNF added=1.08; natural TNF, no Ab=0.290; and recombinant TNF, no Ab=0.500.

FIG. **12** is a graph showing in vitro neutralization of TNF-induced ELAM-1 expression by chimeric A2. The control is a chimeric mouse/human IgG1 anti-CD4 antibody.

FIG. **13** is an amino acid sequence of human TNF as SEQ ID NO:1.

FIGS. **14**A–B FIG. **14**A is a graphical representation of epitope mapping of chimeric mAb cA2 indicating relative binding of cA2 to human TNF peptide pins. FIG. **14**B is a graphical representation of epitope mapping of chimeric mAb cA2 indicating relative binding of cA2 to human TNF peptide pins in the presence of human TNF.

FIG. **15** is an amino acid sequence of human TNF showing sequences having portions of epitopes recognized by cA2, corresponding to portions of amino acids 59–80 and/or 87–108 of SEQ ID NO:1.

FIGS. **16**A–**16**B FIG. **16**A is a nucleic acid sequence (SEQ ID NO:2) and corresponding amino acid sequence (SEQ ID NO:3) of a cloned cA2 light chain variable region. FIG. **16**B is a nucleic acid sequence (SEQ ID NO:4) and corresponding amino acid sequence (SEQ ID NO:5) of a cloned cA2 heavy chain variable region.

FIG. **17** is a graphical representation of the early morning stiffness for the five patients in group I, and the four patients in group II is plotted as the mean percent of the baseline value versus time. Both groups showed an approximately 80 percent decrease or greater in early morning stiffness, which persisted for greater than 40 days.

FIG. **18** is a graphical representation of the assessment of pain using a visual analogue scale for the five patients in group I, and the four patients in group II, is plotted as the mean percent of the baseline value versus time. Both groups showed an approximately 60 to 80 percent decrease in pain score which persisted for greater than 40 days.

FIG. **19** is a graphical representation of the Ritchie Articular Index, (a scale scored of joint tenderness), is plotted as the mean percent of the baseline value versus time. Both groups showed an approximately 80 percent decrease in the Ritchie Articular Index, which persisted for greater than 40 days.

FIG. **20** is a graphical representation of the number of swollen joints for the five patients in group I and the four patients in Group II is plotted as the mean percent of baseline value versus time. Both groups showed an approximately 70 to 80 percent decrease in swollen joints, which persisted for 30 to 40 days.

FIG. **21** is a graphical representation of the serum C-reactive protein for four to five patients in group I, and three of the for patients in group II, is plotted as the mean percent of the baseline value versus time. Both groups showed an approximately 80 percent reduction in CRP which persisted for 30 to 40 days. The values for patient number 1 and patient number 7 were omitted from the computations on which the plots are based, since these patients did not have elevated CRP values at baseline.

FIG. **22** is a graphical representation of the erythrocyte sedimentation rate for the five patients in group I and three of the patients in group II is plotted as the mean percent of the baseline value versus time. Both groups showed an approximately 40 percent reduction in ESR which persisted for at least 40 days. The data from patient number 9 is omitted from the computations on which the plots were based, since this patient did not have an elevated ESR at baseline.

FIG. **23** is a graphical representation of the index of Disease Activity, (a composite score of several parameters of disease activity), for the five patients in group I, and the four patients in group II, is plotted as the mean percent of the baseline value versus time. Both groups showed a clinically significant reduction in IDA, which persisted for at least 40 days.

FIG. **24** is a graphical representation of swollen joint counts (maximum 28), as recorded by a single observer. Circles represent individual patients and horizontal bars show median values at each time point. The screening time point was within 4 weeks of entry to the study (week 0); data from patient 15 were not included after week 2 (dropout). Significance of the changes, relative to week 0, by Mann-Whitney test, adjusted: week 1, $p>0.05$; week 2, $p<0.02$; weeks 3–4, $p<0.002$; weeks 6–8, $p<0.001$.

FIGS. **26**A–B is a graphical representation of levels of serum C-reactive protein (CRP)-Serum CRP (normal range 0–10 mg/liter), measured by nephelometry. Circles represent individual patients and horizontal bars show median values at each time point. The screening time point was within 4 weeks of entry to the study (week 0); data from patient 15 were not included after week 2 (dropout). Significance of the changes, relative to week 0, by Mann-Whitney test, adjusted: week 1, $p<0.001$; week 2, $p<0.003$; week 3, $p<0.002$; week 4, $p<0.02$; week 6,8, $p<0.001$. FIG. **25**B is a schematic illustration of the construction of the vectors used to express the heavy chain of the immunoreceptors.

FIGS. **26**A–B is a schematic illustration of the genes encoding TNF receptor/IgG fusion proteins and the gene encoding the truncated light chain. The gene encoding Ig heavy chain (IgH) fusion proteins had the same basic structure as the naturally occurring, rearranged Ig genes except that the Ig variable region coding sequence was replaced with TNF receptor coding sequence. Except for the TNF receptor coding sequences and a partial human K sequence derived by modifying the murine J region coding sequence in the cM-T412 IgH gene by PCT mutagenesis, the entire genomic fragment shown originated from the cM-T412 chimeric mouse/human IgH gene. Looney et al., *Hum. Antibody Hybrid.* 3:191–200 (1992). The region deleted in the genes encoding p55-sf3 and p75P-sf3 is marked in the figure. The JC_K gene, encoding a truncated Ig Kappa light chain, was constructed by deleting the variable region coding sequence from the cM-T412 chimeric mouse/human Ig Kappa gene (Looney, infra) and using PCR mutagenesis to change the murine J sequence to a partial human J sequence. THe p55-light chain fusion in p55-df2 was made by inserting the p55 coding sequence into the EcoRV site in the JC_K gene. Tracey et al., *Nature* 330:662–666 (1987). FIG. **26**B is a schematic illustration of several immunoreceptor molecules of the present invention. The blackened ovals each represent a domain of the IgG1 constant region. THe circles represent the truncated light chain. Small circles adjacent to a p55 or p75 subunit mark the positions of human J sequence. The incomplete circles in p75-sf2 and -sf3 are to illustrate that the C-terminal 53 amino acids of the p75 extracellular domain were deleted. Lines between subunits represent disulfide bonds.

FIG. **27** is a schematic illustration of the construction of a cM-T412 heavy chain so that it has a unique cloning site for insertion of foreign genes such as p55 and p75.

FIG. **28** is a schematic illustration of the construction of the vectors used to express the heavy chain of the immunoreceptors.

FIG. **29** is a schematic illustration of the construction of a cM-T412 light chain so that it has a unique cloning site for insertion of foreign genes such as p55 and p75.

FIG. **30** is a schematic illustration of the construction of the vectors used to express the light chain of the immunoreceptors.

FIGS. **31**A–C shows graphical representations of fusion proteins protected WEHI 164 cells from TNFβ with actinomycin D and then incubated in 2 ng/ml TNFα with varying concentrations of TNFf overnight at 37° C. Cell viability was determined by measuring their uptake of MTT dye. FIG. **31**A shows p55 fusion proteins. FIG. **31**B shows p75 fusion proteins. FIG. **31**C shows comparison of the protective ability of the non-fusion form of p55 (p55-nf) to p55-sf2.

FIG. **32** is a graphical represation of data showing fusion proteins also effectively protect WEHI 164 cells from TNFβ cytotoxicity.

FIGS. **33**A–H are graphical representations of analyses of binding between the various fusion proteins and TNFα by saturation binding (FIGS. **33**A–B) and Scatchard analysis (FIGS. **33**C–H). A microtiter plate was coated with excess goat anti-Fc polyclonal antibody and incubated with 10 ng/ml of fusion protein in TBST buffer (10 mM Tris-HCl, pH 7.8, 150 mM NaCl, 0.05% Tween-20) for 1 hour. Varying amounts of ^{125}I labeled TNFα (specific activity—34.8 μCi/μg) was then incubated with the captured fusion protein in PBS (10 mM Na Phosphate, pH 7.0, 150 mM NaCl) with 1% bovine serum albumin for 2 hours. Unbound TNFα was washed away with four washes in PBS and the cpm bound was quantitated using a y-counter. All samples were analyzed in triplicate. The slope of the lines in (FIGS. **33**C–H) represent the affinity constant, K_a. The dissociation constant (K_d) values (see Table I) were derived using the equation $K_d=1/K_a$.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Tumor necrosis factor (TNF) has been discovered to mediate or be involved in many pathologies, such as, but not limited to bacterial, viral or parasitic infections, chronic inflammatory diseases, autoimmune diseases, malignancies, and/or neurodegenerative diseases. Accordingly, anti-TNF compounds and compositions of the present invention which have neutralizing and/or inhibiting activity against TNF are discovered to provide methods for treating and/or diagnosing such pathologies.

The present invention thus provides anti-TNF compounds and compositions comprising anti-TNF antibodies (Abs) and/or anti-TNF peptides which inhibit and/or neutralize TNF biological activity in vitro, in situ and/or in vivo, as specific for association with neutralizing epitopes of human tumor necrosis factor-alpha (hTNFα) and/or human tumor necrosis factor β (hTNFβ). Such anti-TNF Abs or peptides have utilities for use in research, diagnostic and/or therapeutic methods of the present invention for diagnosing and/or treating animals or humans having pathologies or conditions associated with the presence of a substance reactive with an anti-TNF antibody, such as TNF or metabolic products thereof. Such pathologies can include the generalized or local presence of TNF or related compounds, in amounts and/or concentrations exceeding, or less than, those present in a normal healthy subject, or as related to a pathological condition.

Anti-TNF Antibodies and Methods

The term "antibody" is meant to include polyclonal antibodies, monoclonal antibodies (mAbs), chimeric antibodies, anti-idiotypic (anti-Id) antibodies to antibodies that can be labeled in soluble or bound form, as well as fragments, regions or derivatives thereof, provided by any known technique, such as, but not limited to enzymatic cleavage, peptide synthesis or recombinant techniques. Such anti-TNF antibodies of the present invention are capable of binding portions of TNF that inhibit the binding of TNF to TNF receptors.

Polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of animals immunized with an antigen. A monoclonal antibody contains a substantially homogeneous population of antibodies specific to antigens, which population contains substantially similar epitope binding sites. MAbs may be obtained by methods known to those skilled in the art. See, for example Kohler and Milstein, *Nature* 256:495–497 (1975); U.S. Pat. No. 4,376,110; Ausubel et al, eds., *CURRENT PROTOCOLS IN MOLECULAR BIOLOGY*, Greene Publishing Assoc. and Wiley Interscience, N.Y., (1987, 1992); and Harlow and Lane *ANTIBODIES: A LABORATORY MANUAL* Cold Spring Harbor Laboratory (1988); Colligan et al., eds., *Current Protocols in Immunology*, Greene Publishing Assoc. and Wiley Interscience, N.Y., (1992, 1993), the contents of which references are incoporated entirely herein by reference. Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, GILD and any subclass thereof. A hybridoma producing a mAb of the present invention may be cultivated in vitro, in situ or in vivo. Production of high titers of mAbs in vivo or in situ makes this the presently preferred method of production.

Chimeric antibodies are molecules different portions of which are derived from different animal species, such as those having variable region derived from a murine mAb and a human immunoglobulin constant region, which are primarily used to reduce immunogenicity in application and to increase yields in production, for example, where murine mAbs have higher yields from hybridomas but higher immunogenicity in humans, such that human/murine chimeric mAbs are used. Chimeric antibodies and methods for their production are known in the art (Cabilly et al, *Proc. Natl. Acad. Sci. USA* 81:3273–3277 (1984); Morrison et al., *Proc. Natl. Acad. Sci. USA* 81:6851–6855 (1984); Boulianne et al., *Nature* 312:643–646 (1984); Cabilly et al., European Patent Application 125023 (published Nov. 14, 1984); Neuberger et al., *Nature* 314:268–270 (1985); Taniguchi et al., European Patent Application 171496 (published Feb. 19, 1985); Morrison et al., European Patent Application 173494 (published Mar. 5, 1986); Neuberger et al., PCT Application WO 86/01533, (published Mar. 13, 1986); Kudo et al., European Patent Application 184187 (published Jun. 11, 1986); Morrison et al., European Patent Application 173494 (published Mar. 5, 1986); Sahagan et al., J. Immunol. 137:1066–1074 (1986); Robinson et al., International Patent Publication #PCT/US86/02269 (published May 7, 1987); Liu et al., Proc. Natl. Acad. Sci. USA 84:3439–3443 (1987); Sun et al., Proc. Natl. Acad. Sci. USA 84:214–218 (1987); Better et al., Science 240:1041–1043 (1988); and Harlow and Lane ANTIBODIES: A LABORATORY MANUAL Cold Spring Harbor Laboratory (1988)). These references are entirely incorporated herein by reference.

An anti-idiotypic (anti-Id) antibody is an antibody which recognizes unique determinants generally associated with the antigen-binding site of an antibody. An Id antibody can be prepared by immunizing an animal of the same species and genetic type (e.g., mouse strain) as the source of the mAb with the mAb to which an anti-Id is being prepared. The immunized animal will recognize and respond to the idiotypic determinants of the immunizing antibody by producing an antibody to these idiotypic determinants (the anti-Id antibody). See, for example, U.S. Pat. No. 4,699,880, which is herein entirely incorporated by reference.

The anti-Id antibody may also be used as an "immunogen" to induce an immune response in yet another animal, producing a so-called anti-anti-Id antibody. The anti-anti-Id may be epitopically identical to the original mAb which induced the anti-Id. Thus, by using antibodies to the idiotypic determinants of a mAb, it is possible to identify other clones expressing antibodies of identical specificity.

Anti-TNF antibodies of the present invention can include at least one of a heavy chain constant region (H_c), a heavy chain variable region (H_v), a light chain variable region (L_v) and a light chain constant regions (L_c), wherein a polyclonal Ab, monoclonal Ab, fragment and/or regions thereof include at least one heavy chain variable region (H_v) or light chain variable region (L_v) which binds a portion of a TNF and inhibits and/or neutralizes at least one TNF biological activity.

Preferred antibodies of the present invention are high affinity human-murine chimeric anti-TNF antibodies, and fragments or regions thereof, that have potent inhibiting and/or neutralizing activity in vivo against human TNFα. Such antibodies and chimeric antibodies can include those generated by immunization using purified recombinant hTNFα (SEQ ID NO:1) or peptide fragments thereof. Such fragments can include epitopes of at least 5 amino acids of residues 87–107, or a combination of both of 59–80 and 87–108 of hTNFα (as these corresponding amino acids of SEQ ID NO:1). Additionally, preferred antibodies, fragments and regions of anti-TNF antibodies of the present invention do not recognize amino acids from at least one of amino acids 11–13, 37–42, 49–57 or 155–157 of hTNFα (of SEQ ID NO:1).

Preferred anti-TNF mAbs are also those which will competitively inhibit in vivo the binding to human TNFα of anti-TNFα murine mAb A2, chimeric mAb cA2, or an antibody having substantially the same specific binding characteristics, as well as fragments and regions thereof. Preferred antibodies of the present invention are those that bind epitopes recognized by A2 and cA2, which are included in amino acids 59–80 and/or 87–108 of hTNFα (as these corresponding amino acids of SEQ ID NO:1), such that the epitopes consist of at least 5 amino acids which comprise at least one amino acid from the above portions of human TNFα.

Preferred methods for determining mAb specificity and affinity by competitive inhibition can be found in Harlow, et al., *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1988), Colligan et al., eds., *Current Protocols in Immunology*, Greene Publishing Assoc. and Wiley Interscience, N.Y., (1992, 1993), and Muller, *Meth. Enzymol.* 92:589–601 (1983), which references are entirely incorporated herein by reference.

The techniques to raise antibodies of the present invention to small peptide sequences that recognize and bind to those sequences in the free or conjugated form or when presented as a native sequence in the context of a large protein are well known in the art. Such antibodies include murine, murine human and human-human antibodies produced by hybridoma or recombinant techniques known in the art.

As used herein, the term "antigen binding region" refers to that portion of an antibody molecule which contains the amino acid residues that interact with an antigen and confer on the antibody its specificity and affinity for the antigen. The antibody region includes the "framework" amino acid residues necessary to maintain the proper conformation of the antigen-binding residues.

Preferably, the antigen binding region will be of murine origin. In other embodiments, the antigen binding region can be derived from other animal species, in particular rodents such as rabbit, rat or hamster.

The antigen binding region of the chimeric antibody of the present invention is preferably derived from a non-human antibody specific for human TNF. Preferred sources for the DNA encoding such a non-human antibody include cell lines which produce antibody, preferably hybrid cell lines commonly known as hybridomas. A preferred hybridoma is the A2 hybridoma cell line.

An "antigen" is a molecule or a portion of a molecule capable of being bound by an antibody which is additionally capable of inducing an animal to produce antibody capable of binding to an epitope of that antigen. An antigen can have one or more than one epitope. The specific reaction referred to above is meant to indicate that the antigen will react, in a highly selective manner, with its corresponding antibody and not with the multitude of other antibodies which can be evoked by other antigens. Preferred antigens that bind antibodies, fragments and regions of anti-TNF antibodies of the present invention include at least 5 amino acids comprising at least one of amino acids residues 87–108 or both residues 59–80 and 87–108 of hTNFα (of SEQ ID NO:1). Preferred antigens that bind antibodies, fragments and regions of anti-TNF antibodies of the present invention do not include amino acids of amino acids 11–13, 37–42, 49–57 or 155–157 of hTNFα (SEQ ID NO:1)

Particular peptides which can be used to generate antibodies of the present invention can include combinations of amino acids selected from at least residues 87–108 or both residues 59–80 and 87–108, which are combined to provide an epitope of TNF that is bound by anti-TNF antibodies, fragments and regions thereof, and which binding provided anti-TNF biological activity. Such epitopes include at least 1–5 amino acids and less than 22 amino acids from residues 87–108 or each of residues 59–80 and 87–108, which in combination with other amino acids of TNF provide epitopes of at least 5 amino acids in length.

TNF residues 87–108 or both residues 59–80 and 87–108 of TNF (of SEQ ID NO:1), fragments or combinations of peptides containing therein are useful as immunogens to raise antibodies that will recognize peptide sequences presented in the context of the native TNF molecule.

The term "epitope" is meant to refer to that portion of any molecule capable of being recognized by and bound by an antibody at one or more of the Ab's antigen binding region. Epitopes usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and have specific three dimensional structural characteristics as well as specific charge characteristics. By "inhibiting and/or neutralizing epitope" is intended an epitope, which, when bound by an antibody, results in loss of biological activity of the molecule or organism containing the epitope, in vivo, in vitro or in situ, more preferably in vivo, including binding of TNF to a TNF receptor.

Epitopes recognized by antibodies, and fragments and regions thereof, of the present invention can include 5 or more amino acids comprising at least one amino acid of each or both of the following amino acid sequences of TNF, which provide a topographical or three dimensional epitope of TNF which is recognized by, and/or binds with anti-TNF activity, an antibody, and fragments, and variable regions thereof, of the present invention:

59–80: Tyr-Ser-Gln-Val-Leu-Phe-Lys-Gly-Gln-Gly-Cys-Pro-Ser-Thr-His-Val-Leu-Leu-Thr-His-Thr-Ile (AA 59–80 of SEQ ID NO:1); and

87–108: Tyr-Gln-Thr-Lys-Val-Asn-Leu-Leu-Ser-Ala-Ile-Lys-Ser-Pro-Cys-Gln-Arg-Glu-Thr-Pro-Gl u-Gly (AA 87–108 of SEQ ID NO:1).

Preferred antibodies, fragments and regions of anti-TNF antibodies of the present invention recognize epitopes including 5 amino acids comprising at least one amino acid from amino acids residues 87–108 or both residues 59–80 and 87–108 of hTNFα (of SEQ ID NO:1). Preferred antibodies, fragments and regions of anti-TNF antibodies of the present invention do not recognize epitopes from at least one of amino acids 11–13, 37–42, 49–57 or 155–157 of hTNFα (of SEQ ID NO:1). In a preferred embodiment, the epitope comprises at least 2 amino acids from residues 87–108 or both residues 59–80 and 87–108 of hTNFα (of SEQ ID NO:1). In another preferred embodiment, the epitope comprises at least 3 amino acids from residues 59–80 and 87–108 of hTNFα (of SEQ ID NO:1). In another preferred embodiment, the epitope comprises at least 4 amino acids from residues 87–108 or both residues 59–80 and 87–108 of hTNFα (of SEQ ID NO:1). In another preferred embodiment, the epitope comprises at least 5 amino acids from residues 87–108 or both residues 59–80 and 87–108 of hTNFα (of SEQ ID NO:1). In another preferred embodiment, the epitope comprises at least 6 amino acids from residues 87–108 or both residues 59–80 and 87–108 of hTNFα (of SEQ ID NO:1). In another preferred embodiment, the epitope comprises at least 7 amino acids from residues 87–108 or both residues 59–80 and 87–108 of hTNFα (of SEQ ID NO:1).

As used herein, the term "chimeric antibody" includes monovalent, divalent or polyvalent immunoglobulins. A monovalent chimeric antibody is a dimer (HL)) formed by a chimeric H chain associated through disulfide bridges with a chimeric L chain. A divalent chimeric antibody is tetramer (H_2L_2) formed by two HL dimers associated through at least one disulfide bridge. A polyvalent chimeric antibody can also be produced, for example, by employing a CH region that aggregates (e.g., from an IgM H chain, or μ chain).

Murine and chimeric antibodies, fragments and regions of the present invention comprise individual heavy (H) and/or light (L) immunoglobulin chains. A chimeric H chain comprises an antigen binding region derived from the H chain of a non-human antibody specific for TNF, which is linked to at least a portion of a human H chain C region (C_H), such as CH_1 or CH_2.

A chimeric L chain according to the present invention, comprises an antigen binding region derived from the L chain of a non-human antibody specific for TNF, linked to at least a portion of a human L chain C region (C_L).

Antibodies, fragments or derivatives having chimeric H chains and L chains of the same or different variable region binding specificity, can also be prepared by appropriate association of the individual polypeptide chains, according to known method steps, e.g., according to Ausubel infra, Harlow infra, and Colligan infra, the contents of which references are incoporated entirely herein by reference.

With this approach, hosts expressing chimeric H chains (or their derivatives) are separately cultured from hosts expressing chimeric L chains (or their derivatives), and the immunoglobulin chains are separately recovered and then associated. Alternatively, the hosts can be co-cultured and the chains allowed to associate spontaneously in the culture medium, followed by recovery of the assembled immunoglobulin, fragment or derivative.

The hybrid cells are formed by the fusion of a non-human anti-hTNFα antibody-producing cell, typically a spleen cell of an animal immunized against either natural or recombinant human TNF, or a peptide fragment of the human TNFα protein sequence. Alternatively, the non-human anti-TNFα antibody-producing cell can be a B lymphocyte obtained from the blood, spleen, lymph nodes or other tissue of an animal immunized with TNF.

The second fusion partner, which provides the immortalizing function, can be lymphoblastoid cell or a plasmacytoma or myeloma cell, which is not itself an antibody producing cell, but is malignant. Preferred fusion partner cells include the hybridoma SP2/0-Ag14, abbreviated as SP2/0 (ATCC CRL1581) and the myeloma P3X63Ag8 (ATCC TIB9), or its derivatives. See, e.g, Ausubel infra, Harlow infra, and Colligan infra, the contents of which references are incoporated entirely herein by reference.

Murine hybridomas which produce mAb specific for human TNFα or TNFβ are formed by the fusion of a mouse fusion partner cell, such as SP2/0, and spleen cells from mice immunized against purified hTNFα, recombinant hTNFα, natural or synthetic TNF peptides, including peptides including 5 or more amino acids selected from residues 59–80, and 87–108 of TNF (of SEQ ID NO:1) or other biological preparations containing TNF. To immunize the mice, a variety of different conventional protocols can be followed. For example, mice can receive primary and boosting immunizations of TNF.

The antibody-producing cell contributing the nucleotide sequences encoding the antigen-binding region of the chimeric antibody of the present invention can also be produced by transformation of a non-human, such as a primate, or a human cell. For example, a B lymphocyte which produces anti-TNF antibody can be infected and transformed with a virus such as Epstein-Barr virus to yield an immortal anti-TNF producing cell (Kozbor et al. *Immunol. Today* 4:72–79 (1983)). Alternatively, the B lymphocyte can be transformed by providing a transforming gene or transforming gene product, as is well-known in the art. See, e.g, Ausubel infra, Harlow infra, and Colligan infra, the contents of which references are incoporated entirely herein by reference.

Antibody Production Using Hybridomas

The cell fusions are accomplished by standard procedures well known to those skilled in the field of immunology. Fusion partner cell lines and methods for fusing and selecting hybridomas and screening for mAbs are well known in the art. See, e.g, Ausubel infra, Harlow infra, and Colligan infra, the contents of which references are incoporated entirely herein by reference.

The hTNFα-specific murine or chimeric mAb of the present invention can be produced in large quantities by injecting hybridoma or transfectoma cells secreting the antibody into the peritoneal cavity of mice and, after appropriate time, harvesting the ascites fluid which contains a high titer of the mAb, and isolating the mAb therefrom. For such in vivo production of the mAb with a non-murine hybridoma (e.g., rat or human), hybridoma cells are preferably grown in irradiated or athymic nude mice. Alternatively, the antibodies can be produced by culturing hybridoma or transfectoma cells in vitro and isolating secreted mAb from the cell culture medium or recombinantly, in eukaryotic or prokaryotic cells.

In a preferred embodiment, the antibody is a MAb which binds amino acids of an epitope of TNF, which antibody is designated A2, rA2 or cA2, which is produced by a hybridoma or by a recombinant host. In another preferred embodiment, the antibody is a chimeric antibody which recognizes an epitope recognized by A2. In a more preferred embodiment, the antibody is a chimeric antibody designated as chimeric A2 (cA2).

As examples of antibodies according to the present invention, murine mAb A2 of the present invention is produced by a cell line designated c134A. Chimeric anti-

body cA2 is produced by a cell line designated c168A. Cell line c134A is deposited as a research cell bank in the Centocor Cell Biology Services Depository, and cell line c168A(RCB) is deposited as a research cell bank in the Centocor Corporate Cell Culture Research and Development Depository, both at Centocor, 200 Great Valley Parkway, Malvern, Pa., 19355. The c168A cell line is also deposited at Centocor BV, Leiden, The Netherlands.

The invention also provides for "derivatives" of the murine or chimeric antibodies, fragments, regions or derivatives thereof, which term includes those proteins encoded by truncated or modified genes to yield molecular species functionally resembling the immunoglobulin fragments. The modifications include, but are not limited to, addition of genetic sequences coding for cytotoxic proteins such as plant and bacterial toxins. The fragments and derivatives can be produced from any of the hosts of this invention. Alternatively, anti-TNF antibodies, fragments and regions can be bound to cytotoxic proteins or compounds in vitro, to provide cytotoxic anti-TNF antibodies which would selectively kill cells having TNF receptors.

Fragments include, for example, Fab, Fab', $F(ab')_2$ and Fv. These fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and can have less non-specific tissue binding than an intact antibody (Wahl et al., *J. Nucl. Med.* 24:316–325 (1983)). These fragments are produced from intact antibodies using methods well known in the art, for example by proteolytic cleavage with enzymes such as papain (to produce Fab fragments) or pepsin (to produce $F(ab')_2$ fragments).

The identification of these antigen binding regions and/or epitopes recognized by mabs of the present invention provides the information necessary to generate additional monoclonal antibodies with similar binding characteristics and therapeutic or diagnostic utility that parallel the embodiments of this application.

In a preferred embodiment, the amino acids of the epitope are not of at least one of amino acids 11–13, 37–42, 49–57 and 155–157 of hTNFα (of SEQ ID NO:1).

Unexpectedly, anti-TNF antibodies or peptides of the present invention can block the action of TNF-α without binding to the putative receptor binding locus such as is presented by Eck and Sprang (J. Biol. Chem. 264(29), 17595–17605 (1989), as amino acids 11–13, 37–42, 49–57 and 155–157 of hTNFα (of SEQ ID NO:1).

Recombinant Expression of Anti-TNF Antibodies

Recombinant murine or chimeric murine-human or human-human antibodies that inhibit TNF and bind an epitope included in the amino acid sequences residues 87–108 or both residues 59–80 and 87–108 of hTNFα (of SEQ ID NO:1), can be provided according to the present invention using known techniques based on the teaching provided herein. See, e.g., Ausubel et al., eds. *Current Protocols in Molecular Biology*, Wiley Interscience, N.Y. (1987, 1992, 1993); and Sambrook et al. *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press (1989), the entire contents of which are incorporated herein by reference.

The DNA encoding an anti-TNF antibody of the present invention can be genomic DNA or cDNA which encodes at least one of the heavy chain constant region (H_c), the heavy chain variable region (H_v), the light chain variable region (L_v) and the light chain constant regions (L_c). A convenient alternative to the use of chromosomal gene fragments as the source of DNA encoding the murine V region antigen-binding segment is the use of cDNA for the construction of chimeric immunoglobulin genes, e.g., as reported by Liu et al. (*Proc. Natl. Acad. Sci., USA* 84:3439 (1987) and *J. Immunology* 139:3521 (1987), which references are hereby entirely incorporated herein by reference. The use of cDNA requires that gene expression elements appropriate for the host cell be combined with the gene in order to achieve synthesis of the desired protein. The use of cDNA sequences is advantageous over genomic sequences (which contain introns), in that cDNA sequences can be expressed in bacteria or other hosts which lack appropriate RNA splicing systems.

For example, a cDNA encoding a murine V region antigen-binding segment having anti-TNF activity can be provided using known methods based on the use of the DNA sequence presented in FIG. **16**A (SEQ ID NO:2). Alternatively, a cDNA encoding a murine C region antigen-binding segment having anti-TNF activity can be provided using known methods based on the use of the DNA sequence presented in FIG. **16**B (SEQ ID NO:3). Probes that bind a portion of the DNA sequence presented in FIG. **16**A or **16**B can be used to isolate DNA from hybridomas expressing TNF antibodies, fragments or regions, as presented herein, according to the present invention, by known methods.

Oligonucleotides representing a portion of the variable region presented in FIG. **16**A or **16**B sequence are useful for screening for the presence of homologous genes and for the cloning of such genes encoding variable or constant regions of an anti-TNF antibody. Such probes preferably bind to portions of sequences according to FIG. **17**A or **17**B which encode light chain or heavy chain variable regions which bind an activity inhibiting epitope of TNF, especially an epitope of at least 5 amino acids of residues 87–108 or a combination of residues 59–80 and 87–108 (of SEQ ID NO:1).

Such techniques for synthesizing such oligonucleotides are well known and disclosed by, for example, Wu, et al., *Prog. Nucl. Acid. Res. Molec. Biol.* 21:101–141 (1978)), and Ausubel et al, eds. *Current Protocols in Molecular Biology*, Wiley Interscience (1987, 1993), the entire contents of which are herein incorporated by reference.

Because the genetic code is degenerate, more than one codon can be used to encode a particular amino acid (Watson, et al., infra). Using the genetic code, one or more different oligonucleotides can be identified, each of which would be capable of encoding the amino acid. The probability that a particular oligonucleotide will, in fact, constitute the actual XXX-encoding sequence can be estimated by considering abnormal base pairing relationships and the frequency with which a particular codon is actually used (to encode a particular amino acid) in eukaryotic or prokaryotic cells expressing an anti-TNF antibody or fragment. Such "codon usage rules" are disclosed by Lathe, et al., *J. Molec. Biol.* 183:1–12 (1985). Using the "codon usage rules" of Lathe, a single oligonucleotide, or a set of oligonucleotides, that contains a theoretical "most probable" nucleotide sequence capable of encoding anti-TNF variable or constant region sequences is identified.

Although occasionally an amino acid sequence can be encoded by only a single oligonucleotide, frequently the amino acid sequence can be encoded by any of a set of similar oligonucleotides. Importantly, whereas all of the members of this set contain oligonucleotides which are capable of encoding the peptide fragment and, thus, potentially contain the same oligonucleotide sequence as the gene which encodes the peptide fragment, only one member of the set contains the nucleotide sequence that is identical to the nucleotide sequence of the gene. Because this member is present within the set, and is capable of hybridizing to DNA

even in the presence of the other members of the set, it is possible to employ the unfractionated set of oligonucleotides in the same manner in which one would employ a single oligonucleotide to clone the gene that encodes the protein.

The oligonucleotide, or set of oligonucleotides, containing the theoretical "most probable" sequence capable of encoding an anti-TNF antibody or fragment including a variable or constant region is used to identify the sequence of a complementary oligonucleotide or set of oligonucleotides which is capable of hybridizing to the "most probable" sequence, or set of sequences. An oligonucleotide containing such a complementary sequence can be employed as a probe to identify and isolate the variable or constant region anti-TNF gene (Sambrook et al., infra).

A suitable oligonucleotide, or set of oligonucleotides, which is capable of encoding a fragment of the variable or constant anti-TNF region (or which is complementary to such an oligonucleotide, or set of oligonucleotides) is identified (using the above-described procedure), synthesized, and hybridized by means well known in the art, against a DNA or, more preferably, a cDNA preparation derived from cells which are capable of expressing anti-TNF antibodies or variable or constant regions thereof. Single stranded oligonucleotide molecules complementary to the "most probable" variable or constant anti-TNF region peptide coding sequences can be synthesized using procedures which are well known to those of ordinary skill in the art (Belagaje, et al., *J. Biol. Chem.* 254:5765–5780 (1979); Maniatis, et al., *In: Molecular Mechanisms in the Control of Gene Expression*, Nierlich, et al., Eds., Acad. Press, NY (1976); Wu, et al., *Prog. Nucl. Acid Res. Molec. Biol.* 21:101–141 (1978); Khorana, *Science* 203:614–625 (1979)). Additionally, DNA synthesis can be achieved through the use of automated synthesizers. Techniques of nucleic acid hybridization are disclosed by Sambrook et al. (infra), and by Haymes, et al. (In: *Nucleic Acid Hybridization, A Practical Approach*, IRL Press, Washington, D.C. (1985)), which references are herein incorporated by reference. Techniques such as, or similar to, those described above have successfully enabled the cloning of genes for human aldehyde dehydrogenases (Hsu, et al., *Proc. Natl. Acad. Sci. USA* 82:3771–3775 (1985)), fibronectin (Suzuki, et al., *Eur. Mol. Biol. Organ. J.* 4:2519–2524 (1985)), the human estrogen receptor gene (Walter, et al., *Proc. Natl. Acad. Sci. USA* 82:7889–7893 (1985)), tissue-type plasminogen activator (Pennica, et al., *Nature* 301:214–221 (1983)) and human term placental alkaline phosphatase complementary DNA (Kam, et al., *Proc. Natl. Acad. Sci. USA* 82:8715–8719 (1985)).

In an alternative way of cloning a polynucleotide encoding an anti-TNF variable or constant region, a library of expression vectors is prepared by cloning DNA or, more preferably, cDNA (from a cell capable of expressing an anti-TNF antibody or variable or constant region) into an expression vector. The library is then screened for members capable of expressing a protein which competitively inhibits the binding of an anti-TNF antibody, such as A2 or cA2, and which has a nucleotide sequence that is capable of encoding polypeptides that have the same amino acid sequence as anti-TNF antibodies or fragments thereof. In this embodiment, DNA, or more preferably cDNA, is extracted and purified from a cell which is capable of expressing an anti-TNF antibody or fragment. The purified cDNA is fragmentized (by shearing, endonuclease digestion, etc.) to produce a pool of DNA or cDNA fragments. DNA or cDNA fragments from this pool are then cloned into an expression vector in order to produce a genomic library of expression vectors whose members each contain a unique cloned DNA or cDNA fragment such as in a lambda phage library, expression in prokaryotic cell (e.g., bacteria) or eukaryotic cells, (e.g., mammalian, yeast, insect or fungus). See, e.g., Ausubel, infra, Harlow, infra, Colligan, infra; Nyyssonen et al. *Bio/Technology* 11:591–595 (Can 1993); Marks et al., *Bio/Technology* 11:1145–1149 (October 1993). Once nucleic acid encoding such variable or constant anti-TNF regions is isolated, the nucleic acid can be appropriately expressed in a host cell, along with other constant or variable heavy or light chain encoding nucleic acid, in order to provide recombinant MAbs that bind TNF with inhibitory activity. Such antibodies preferably include a murine or human anti-TNF variable region which contains a framework residue having complimentarily determining residues which are responsible for antigen binding. In a preferred embodiment, an anti-TNF variable light or heavy chain encoded by a nucleic acid as described above binds an epitope of at least 5 amino acids including residues 87–108 or a combination of residues 59–80 and 87–108 of hTNF (of SEQ ID NO:1).

Human genes which encode the constant (C) regions of the murine and chimeric antibodies, fragments and regions of the present invention can be derived from a human fetal liver library, by known methods. Human C regions genes can be derived from any human cell including those which express and produce human inmuunoglobulins. The human C_H region can be derived from any of the known classes or isotypes of human H chains, including gamma, μ, α, δ or ϵ, and subtypes thereof, such as G1, G2, G3 and G4. Since the H chain isotype is responsible for the various effector functions of an antibody, the choice of C_H region will be guided by the desired effector functions, such as complement fixation, or activity in antibody-dependent cellular cytotoxicity (ADCC). Preferably, the C_H region is derived from gamma 1 (IgG1), gamma 3 (IgG3), gamma 4 (IgG4), or μ (IgM).

The human C_L region can be derived from either human L chain isotype, kappa or lambda.

Genes encoding human immunoglobulin C regions are obtained from human cells by standard cloning techniques (Sambrook, et al. (*Molecular Cloning: A Laboratory Manual*, 2nd Edition, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (1989) and Ausubel et al, eds. *Current Protocols in Molecular Biology* (1987–1993)). Human C region genes are readily available from known clones containing genes representing the two classes of L chains, the five classes of H chains and subclasses thereof. Chimeric antibody fragments, such as $F(ab')_2$ and Fab, can be prepared by designing a chimeric H chain gene which is appropriately truncated. For example, a chimeric gene encoding an H chain portion of an $F(ab')_2$ fragment would include DNA sequences encoding the CH_1 domain and hinge region of the H chain, followed by a translational stop codon to yield the truncated molecule.

Generally, the murine, human or murine and chimeric antibodies, fragments and regions of the present invention are produced by cloning DNA segments encoding the H and L chain antigen-binding regions of a TNF-specific antibody, and joining these DNA segments to DNA segments encoding C_H and C_L regions, respectively, to produce murine, human or chimeric immunoglobulin-encoding genes.

Thus, in a preferred embodiment, a fused chimeric gene is created which comprises a first DNA segment that encodes at least the antigen-binding region of non-human origin, such as a functionally rearranged V region with joining (J) segment, linked to a second DNA segment encoding at least a part of a human C region.

Therefore, cDNA encoding the antibody V and C regions, the method of producing the chimeric antibody according to the present invention involves several steps, outlined below:

1. isolation of messenger RNA (mRNA) from the cell line producing an anti-TNF antibody and from optional additional antibodies supplying heavy and light constant regions; cloning and cDNA production therefrom;
2. preparation of a full length cDNA library from purified mRNA from which the appropriate V and/or C region gene segments of the L and H chain genes can be: (i) identified with appropriate probes, (ii) sequenced, and (iii) made compatible with a C or V gene segment from another antibody for a chimeric antibody;
3. Construction of complete H or L chain coding sequences by linkage of the cloned specific V region gene segments to cloned C region gene, as described above;
4. Expression and production of L and H chains in selected hosts, including prokaryotic and eukaryotic cells to provide murine-murine, human-murine, human-human or human murine antibodies.

One common feature of all immunoglobulin H and L chain genes and their encoded mRNAs is the J region. H and L chain J regions have different sequences, but a high degree of sequence homology exists (greater than 80%) among each group, especially near the C region. This homology is exploited in this method and consensus sequences of H and L chain J regions can be used to design oligonucleotides for use as primers for introducing useful restriction sites into the J region for subsequent linkage of V region segments to human C region segments.

C region cDNA vectors prepared from human cells can be modified by site-directed mutagenesis to place a restriction site at the analogous position in the human sequence. For example, one can clone the complete human kappa chain C (C_k) region and the complete human gamma-1 C region ($C_{gamma\text{-}1}$). In this case, the alternative method based upon genomic C region clones as the source for C region vectors would not allow these genes to be expressed in bacterial systems where enzymes needed to remove intervening sequences are absent. Cloned V region segments are excised and ligated to L or H chain C region vectors. Alternatively, the human $C_{gamma\text{-}1}$ region can be modified by introducing a termination codon thereby generating a gene sequence which encodes the H chain portion of an Fab molecule. The coding sequences with linked V and C regions are then transferred into appropriate expression vehicles for expression in appropriate hosts, prokaryotic or eukaryotic.

Two coding DNA sequences are said to be "operably linked" if the linkage results in a continuously translatable sequence without alteration or interruption of the triplet reading frame. A DNA coding sequence is operably linked to a gene expression element if the linkage results in the proper function of that gene expression element to result in expression of the coding sequence.

Expression vehicles include plasmids or other vectors. Preferred among these are vehicles carrying a functionally complete human C_H or C_L chain sequence having appropriate restriction sites engineered so that any V_H or V_L chain sequence with appropriate cohesive ends can be easily inserted therein. Human C_H or C_L chain sequence-containing vehicles thus serve as intermediates for the expression of any desired complete H or L chain in any appropriate host.

A chimeric antibody, such as a mouse-human or human-human, will typically be synthesized from genes driven by the chromosomal gene promoters native to the mouse H and L chain V regions used in the constructs; splicing usually occurs between the splice donor site in the mouse J region and the splice acceptor site preceding the human C region and also at the splice regions that occur within the human C_H region; polyadenylation and transcription termination occur at native chromosomal sites downstream of the human coding regions.

Non-Limiting Exemplary Chimeric A2 (cA2) Anti-TNF Antibody of the Present Invention

Murine MAbs are undesirable for human therapeutic use, due to a short free circulating serum half-life and the stimulation of a human anti-murine antibody (HAMA) response. A murine-human chimeric anti-human TNFα MAb was developed in the present invention with high affinity, epitope specificity and the ability to neutralize the cytotoxic effects of human TNF. Chimeric A2 anti-TNF consists of the antigen binding variable region of the high-affinity neutralizing mouse anti-human TNF IgG1 antibody, designated A2, and the constant regions of a human IgG1, kappa immunoglobulin. The human IgG1 Fc region is expected to: improve allogeneic antibody effector function; increase the circulating serum half-life; and decrease the immunogenicity of the antibody. A similar murine-human chimeric antibody (chimeric 17-1A) has been shown in clinical studies to have a 6-fold longer in vivo circulation time and to be significantly less immunogenic than its corresponding murine MAb counterpart (LoBuglio et al., *Proc Natl Acad Sci USA* 86: 4220–4224, 1988).

The avidity and epitope specificity of the chimeric A2 is derived from the variable region of the murine A2. In a solid phase ELISA, cross-competition for TNF was observed between chimeric and murine A2, indicating an identical epitope specificity of cA2 and murine A2. The specificity of cA2 for TNF-α was confirmed by its inability to neutralize the cytotoxic effects of lymphotoxin(TNF-β). Chimeric A2 neutralizes the cytotoxic effect of both natural and recombinant human TNF in a dose dependent manner. From binding assays of cA2 and recombinant human TNF, the affinity constant of cA2 was calculated to be $1.8\times10^{9}M^{-1}$.

ANTI-TNF Immunoreceptor Peptides

Immunoreceptor peptides of this invention can bind to TNFα and/or TNFβ. The immunoreceptor comprises covalently attached to at least a portion of the TNF receptor at least one immunoglobulin heavy or light chain. In certain preferred embodiments, the heavy chain constant region comprises at least a portion of CH_1. Specifically, where a light chain is included with an immunoreceptor peptide, the heavy chain must include the area of CH_1 responsible for binding a light chain constant region.

An immunoreceptor peptide of the present invention can preferably comprise at least one heavy chain constant region and, in certain embodiments, at least one light chain constant region, with a receptor molecule covalently attached to at least one of the immunoglobulin chains. Light chain or heavy chain variable regions are included in certain embodiments. Since the receptor molecule can be linked within the interior of an immunoglobulin chain, a single chain can have a variable region and a fusion to a receptor molecule.

The portion of the TNF receptor linked to the immunoglobulin molecule is capable of binding TNFα and/or TNFβ. Since the extracellular region of the TNF receptor binds TNF, the portion attached to the immunoglobulin molecule of the immunoreceptor consists of at least a portion of the extracellular region of the TNF receptor. In certain preferred embodiments, the entire extracellular region of p55 is included. In other preferred embodiments, the entire extracellular region of p75 is included. In further preferred

embodiments, the extracellular region of p75 is truncated to delete at least a portion of a region of O-linked glycosylation and/or a proline-rich region while leaving intact the intramolecular disulfide bridges. Such immunoreceptors comprise at least a portion of a hinge region wherein at least one heavy chain is covalently linked to a truncated p75 extracellular region capable of binding to TNFα or TNFβ or both. Such a truncated molecule includes, for example, sequences 1–178, 1–182 or at least 5 amino acid portions thereof, such as 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, . . . 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290 or any value thereon.

Certain embodiments can also include, for example, the C-terminal half of the hinge region to provide a disulfide bridge between heavy chains where both CH_2 and CH_3 chains are present and CH_1 is absent. Alternatively, for example, the N-terminal half of the hinge region can be included to provide a disulfide bridge with a light chain where only the CH_1 region is present.

In certain preferred embodiments of this invention, the non-immunoglobulin molecule is covalently linked to the N-terminus of at least one CH_1 region. In other preferred embodiments, the non-immunoglobulin molecule is covalently linked to an interior section of at least one heavy and/or light chain region. Thus, a portion of the TNF receptor can be, for example, at the end of the immunoglobulin chain or in the middle of the chain.

Where the TNF receptor is attached to the middle of the immunoglobulin, the immunoglobulin chain can be truncated, for example, to compensate for the presence of foreign amino acids, thus resulting in a fusion molecule of approximately the same length as a natural immunoglobulin chain. Alternatively, for example, the immunoglobulin chain can be present substantially in its entirety, thus resulting in a chain that is longer than the corresponding natural immunoglobulin chain. Additionally, the immunoglobulin molecule can be truncated to result in a length intermediate between the size of the entire chain linked to the receptor molecule and the size of the immunoglobulin chain alone.

In certain preferred embodiments, the heavy chain is an IgG class heavy chain. In other preferred embodiments, the heavy chain is an IgM class heavy chain.

In certain preferred embodiments, the heavy chain further comprises at least about 8 amino acids of a J region.

In certain preferred embodiments, at least a portion of the hinge region is attached to the CH_1 region. For example, where CH_1 and CH_2 are present in the molecule, the entire hinge region is also present to provide the disulfide bridges between the two heavy chain molecules and between the heavy and light chains. Where only CH_1 is present, for example, the molecule need only contain the portion of the hinge region corresponding to the disulfide bridge between the light and heavy chains, such as the first 7 amino acids of the hinge.

It will be understood by one skilled in the art, once armed with the present disclosure, that the immunoreceptor peptides of the invention can be, for example, monomeric or dimeric. For example, the molecules can have only one light chain and one heavy chain or two light chains and two heavy chains.

At least one of the non-immunoglobulin molecules linked to an immunoglobin molecule comprises at least a portion of p55 or at least a portion of p75. The portion of the receptor that is included encompasses the TNF binding site.

In certain preferred embodiments, the non-immunoglobulin molecule comprises at least 5 amino acid segments of sequences 2–159 of p55. In other preferred embodiments, the non-immunoglobulin molecule comprises at least 5 amino acid portions of sequences 1–235 of p75. In further preferred embodiments, the non-immunoglobulin molecule comprises at least 5 amino acid portions of sequences 1–182 of p75. The above 5 amino acid portions can be selected from 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, . . . 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290.

In certain preferred embodiments, each of the two heavy chains and each of the two light chains is linked to a portion of the TNF receptor, thus forming a tetravalent molecule. Such a tetravalent molecule can have, for example, four p55 receptor molecules; two on the two heavy chains and two on the two light chains. Alternatively, a tetravalent molecule can have, for example, a p55 receptor molecule attached to each of the two heavy chains and a p75 receptor molecule attached to each of the two light chains. A tetravalent molecule can also have, for example, p55 receptor attached to the light chains and p75 receptor attached to the heavy chains. Additionally, a tetravalent molecule can have one heavy chain attached to p55, one heavy chain attached to p75, one light chain attached to p75, and one light chain attached to p55. See, for example, the molecules depicted in FIG. **26**A. Further, the molecules can have six receptors attached, for example; two within the heavy chains and four at the ends of the heavy and light chains. Other potential multimers and combinations would also be within the scope of one skilled in the art, once armed with the present disclosure.

In further preferred embodiments, at least one of the heavy chains has a variable region capable of binding to a second target molecule. Such molecules include, for example, CD3, so that one half of a fusion molecule is a monomeric anti-CD3 antibody.

Additionally, in other embodiments of the present invention, the immunoreceptor peptides further include an irrelevant variable region on the light chain and/or heavy chain. Preferably, however, such a region is absent due to the lowered affinity for TNF which can be present due to stearic hindrance.

In certain preferred embodiments, the heavy chain is linked to a non-immunoglobulin molecule capable of binding to a second target molecule, such as a cytotoxic protein, thus creating a part immunoreceptor, part immunotoxin that is capable of killing those cells expressing TNF. Such cytotoxic proteins, include, but are not limited to, Ricin-A, Pseudomonas toxin, Diphtheria toxin and TNF. Toxins conjugated to ligands are known in the art (see, for example, Olsnes, S. et al., *Immunol. Today* 1989, 10, 291–295). Plant and bacterial toxins typically kill cells by disruption the protein synthetic machinery.

The Immunoreceptors of this invention can be conjugated to additional types of therapeutic moieties including, but not limited to, radionuclides, cytotoxic agents and drugs. Examples of radionuclides include ^{212}Bi, ^{131}I, ^{186}Re, and ^{90}Y, which list is not intended to be exhaustive. The radionuclides exert their cytotoxic effect by locally irradiating the cells, leading to various intracellular lesions, as is known in the art of radiotherapy.

Cytotoxic drugs which can be conjugated to the immunoreceptors and subsequently used for in vivo therapy include, but are not limited to, daunorubicin, doxorubicin, methotrexate, and Mitomycin C. Cytotoxic drugs interfere with critical cellular processes including DNA, RNA, and

protein synthesis. For a fuller exposition of these classes of drugs which are known in the art, and their mechanisms of action, see Goodman, A. G., et al., *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 8th Ed., Macmillan Publishing Col, 1990. Katzung, ed., Basic and Clinical Pharmacology, Fifth Edition, p 768–769, 808–809, 896, Appleton and Lange, Norwalk, Conn.

In preferred embodiments, immunoreceptor molecules of the invention are capable of binding with high affinity to a neutralizing epitope of human TNFα or TNFβ in vivo. Preferably, the binding affinity is at least about 1.6×10^{10} M-1. See, for example, Table 1 below. Additionally, in preferred embodiments, immunoreceptor molecules of the invention are capable of neutralizing TNF at an efficiency of about a concentration of less than 130 pM to neutralize 39.2 pM human TNFα. See, for example, Table 1.

TABLE 1

Summary of affinities of different fusion proteins for TNFα

protein	IC_{50}*	Molar ration fp: TNFα at IC_{50}	K_D (pM)
p55-sf2	70	1.8	57
p55-df2	55	1.4	60
p55-sf3	100	2.6	48
p55-nf	36,000	900	n.d.
p75-sf2	130	3.3	33
p75P-sf2	70	1.8	29
p75P-sf3	130	3.3	15

*IC_{50} = concentration of fusion protein required to inhibit 2 ng/ml (39.2 pM) TNFα by 50% .62

Once armed with the present disclosure, one skilled in the art would be able to create fragments of the immunoreceptor peptides of the invention. Such fragments are intended to be within the scope of this invention. For example, once the molecules are isolated, they can be cleaved with protease to generate fragments that remain capable of binding TNF.

Once armed with the present disclosure, one skilled in the art would also be able to create derivatives of the immunoreceptor peptides of the invention. Such derivatives are intended to be within the scope of this invention. For example, amino acids in the immunoreceptor that constitute a protease recognition site can be modified to avoid protease cleavage and thus confer greater stability, such as KEX2 sites.

One skilled in the art, once armed with the present disclosure, would be able to synthesize the molecules of the invention. The immunoreceptor peptides can be constructed, for example, by vector-mediated synthesis, as described in Example XXIV. In general, two expression vectors are preferably used; one for the heavy chain, one for the light chain. A vector for expression an immunoglobulin preferably consists of a promoter linked to the signal sequence, followed by the constant region. The vector additionally preferably contains a gene providing for the selection of transfected cells expressing the construct. In certain preferred embodiments, sequences derived from the J region are also included.

The immunoglobulin gene can be from any vertebrate source, such as murine, but preferably, it encodes an immunoglobulin having a substantial number of sequences that are of the same origin as the eventual recipient of the immunoreceptor peptide. For example, if a human is treated with a molecule of the invention, preferably the immunoglobulin is of human origin.

TNF receptor constructs for linking to the heavy chain can be synthesized, for example, using DNA encoding amino acids present in the cellular domain of the receptor. Putative receptor binding loci of hTNF have been presented by Eck and Sprange, *J. Biol. Chem.* 1989, 264(29), 17595–17605, who identified the receptor binding loci of TNF-α as consisting of amino acids 11–13, 37–42, 49–57 and 155–157. PCT application WO91/02078 (priority date of Aug. 7, 1989) discloses TNF ligands which can bind to monoclonal antibodies having the following epitopes of at least one of 1–20, 56–77, and 108–127; at least two of 1–20, 56–77, 108–127 and 138–149; all of 1–18, 58–65, 115–125 and 138–149; all of 1–18, and 108–128; all of 56–79, 110–127 and 135– or 136–155; all of 1–30, 117–128 and 141–153; all of 1–26, 117–128 and 141–153; all of 22–40, 49–96 or –97, 110–127 and 136–153; all of 12–22, 36–45, 96–105 and 132–157; all of both of 1–20 and 76–90; all of 22–40, 69–97, 105–128 and 135–155; all of 22–31 and 146–157; all of 22–40 and 49–98; at least one of 22–40, 49–98 and 69–97, both of 22–40 and 70–87. Thus, one skilled in the art, once armed with the present disclosure, would be able to create TNF receptor fusion proteins using portions of the receptor that are known to bind TNF.

Advantages of using an immunoglobulin fusion protein (immunoreceptor peptide) of the present invention include one or more of (1) possible increased avidity for multivalent ligands due to the resulting bivalency of dimeric fusion proteins, (2) longer serum half-life, (3) the ability to activate effector cells via the Fc domain, (4) ease of purification (for example, by protein A chromatography), (5) affinity for TNFα and TNFβ and (6) the ability to block TNFα or TNFβ cytotoxicity.

TNF receptor/IgG fusion proteins have shown greater affinity for TNF α in vitro than their monovalent, non-fusion counterparts. These types of fusion proteins, which also bind murine TNF with high affinity, have also been shown to protect mice from lipopolysaccharide-induced endotoxemia. Lesslauer et al., *Eur. J. Immunol.* 1991, 21, 2883–2886; and Ashkenazi et al., *Proc. Natl. Acad. Sci. USA* 1991, 88, 10535–10539. Unlike the molecules of the present invention, the TNF receptor/IgG fusion proteins reported to date have had the receptor sequence fused directly to the hinge domain of IgGs such that the first constant domain (CH_1) of the heavy chain was omitted. Lesslauer et al., *Eur. J. Immunol.* 1991, 21, 2883–2886; Ashkenzi et al., *Proc. Natl. Acad. Sci. USA* 1991, 88, 10535–10539; and Peppel et al., *J. Exp. Med.* 1991, 174, 1483–1489.

While this generally permits secretion of the fusion protein in the absence of an Ig light chain, a major embodiment of the present invention provides for the inclusion of the CH_1 domain, which can confer advantages such as (1) increased distance and/or flexibility between two receptor molecules resulting in greater affinity for TNF, (2) the ability to create a heavy chain fusion protein and a light chain fusion protein that would assemble with each other and dimerize to form a tetravalent (double fusion) receptor molecule, and (3) a tetravalent fusion protein can have increased affinity and/or neutralizing capability for TNF compared to a bivalent (single fusion) molecule.

Unlike other TNF receptor/IgG fusion proteins that have been reported, the fusion proteins of a major embodiment of the present invention include the first constant domain (CH_1) of the heavy chain. The CH_1 domain is largely responsible for interactions with light chains. The light chain, in turn, provides a vehicle for attaching a second set of TNF receptor molecules to the immunoreceptor peptide.

It was discovered using the molecules of the present invention that the p55/light chain fusion proteins and p55/heavy chain fusion proteins would assemble with each other

and dimerize to form an antibody-like molecule that is tetravalent with respect to p55. The resulting tetravalent p55 molecules can confer more protection against, and have greater affinity for, TNFα or TNFβ than the bivalent p55 molecules. Despite the presumed close proximity of the two light chain p55 domains to the heavy chain p55 domains, they do not appear to stereocilia hinder or reduce the affinity for TNF.

Inclusion of the CH_1 domain also meant that secretion of the fusion protein was likely to be inefficient in the absence of light chain. This has been shown to be due to a ubiquitous immunoglobulin binding protein (BiP) that binds to the C_H1 domain of heavy chains that are not assembled with a light chain and sequesters them in the endoplasmic reticulum. Karlsson et al., *J. Immunol. Methods* 1991, 145, 229–240.

In initial experiments, an irrelevant light chain was co-transfected with the p55-heavy chain construct and subsequent analyses showed that the two chains did assemble and that the resulting fusion protein protected WEHI cells from TNFα. However, it was considered likely that the variable region of the irrelevant light chain would stereocilia hinder interactions between the p55 subunits and TNFα. For this reason, a mouse-human chimeric antibody light chain gene was engineered by (1) deleting the variable region coding sequence, and (2) replacing the murine J coding sequence with human J coding sequence. Use of this truncated light chain, which was shown to assemble and disulfide bond with heavy chains, increased the efficiency of TNF inhibition by approximately 30-fold compared to use of a complete irrelevant light chain.

Comparison of the abilities of p75-sf2 and p75P-sf2 to inhibit TNF cytotoxicity indicated that the C-terminal 53 amino acids of the extracellular domain of p75, which defines a region that is rich in proline residues and contains the only sites of O-linked glycosylation, are not necessary for high-affinity binding to TNFα or TNFβ. In fact, the p75P-sf2 construct repeatedly showed higher affinity binding to TNFβ than p75-sf2. Surprisingly, there was no difference observed between the two constructs in their affinity for TNFα.

It is possible that a cell-surface version of p75-P would also bind TNFβ with higher affinity than the complete p75 extracellular domain. A similar region is found adjacent to the transmembrane domain in the low affinity nerve growth factor receptor whose extracellular domain shows the same degree of similarity to p75 as p55 does. Mallett et al., *Immunol. Today* 1991, 12, 220–223.

Two groups have reported that in cell cytotoxicity assays, their p55 fusion protein could be present at a 3-fold (Lesslauer et al., *Eur. J. Immunol.* 1991, 21, 2883–2886) or 6–8 fold (Ashkenazi et al., *Proc. Natl. Acad. Sci. USA* 1991, 88, 10535–10539) lower concentration than their monovalent p55 and still get the same degree of protection, while another group (Peppel et al., *J. Exp. Med.* 1991, 174, 1483–1489) showed that their p55 fusion protein could be present at a 1000-fold lower concentration than monomeric p55. Thus, the prior art has shown unpredictability in the great variability in the efficiency of different fusion proteins.

The molecules of the present invention have demonstrated the same degree of protection against TNF in a 5000-fold lower molar concentration than monomeric p55. (See Table 1.) It is believed that the presence of the CH_1 chain in the molecules of a major embodiment of the present invention can confer greater flexibility to the molecule and avoid stearic hindrance with the binding of the TNF receptor.

Recombinant Expression of Anti-TNF Antibodies and Anti-TNF Peptides.

A nucleic acid sequence encoding at least one anti-TNF peptide or Ab fragment of the present invention may be recombined with vector DNA in accordance with conventional techniques, including blunt-ended or staggered-ended termini for ligation, restriction enzyme digestion to provide appropriate termini, filling in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and ligation with appropriate ligases. Techniques for such manipulations are disclosed, e.g., by Ausubel, infra, Sambrook, infra, entirely incorporated herein by reference, and are well known in the art.

A nucleic acid molecule, such as DNA, is said to be "capable of expressing" a polypeptide if it contains nucleotide sequences which contain transcriptional and translational regulatory information and such sequences are "operably linked" to nucleotide sequences which encode the polypeptide. An operable linkage is a linkage in which the regulatory DNA sequences and the DNA sequence sought to be expressed are connected in such a way as to permit gene expression as anti-TNF peptides or Ab fragments in recoverable amounts. The precise nature of the regulatory regions needed for gene expression may vary from organism to organism, as is well known in the analogous art. See, e.g., Sambrook, supra and Ausubel supra.

The present invention accordingly encompasses the expression of an anti-TNF peptide or Ab fragment, in either prokaryotic or eukaryotic cells, although eukaryotic expression is preferred.

Preferred hosts are bacterial or eukaryotic hosts including bacteria, yeast, insects, fungi, bird and mammalian cells either in vivo, or in situ, or host cells of mammalian, insect, bird or yeast origin. It is preferred that the mammalian cell or tissue is of human, primate, hamster, rabbit, rodent, cow, pig, sheep, horse, goat, dog or cat origin, but any other mammalian cell may be used.

Further, by use of, for example, the yeast ubiquitin hydrolase system, in vivo synthesis of ubiquitin-transmembrane polypeptide fusion proteins may be accomplished. The fusion proteins so produced may be processed in vivo or purified and processed in vitro, allowing synthesis of an anti-TNF peptide or Ab fragment of the present invention with a specified amino terminus sequence. Moreover, problems associated with retention of initiation codon-derived methionine residues in direct yeast (or bacterial) expression may be avoided. Sabin et al., *Bio/Technol.* 7(7): 705–709 (1989); Miller et al., *Bio/Technol.* 7(7): 698–704 (1989).

Any of a series of yeast gene expression systems incorporating promoter and termination elements from the actively expressed genes coding for glycolytic enzymes produced in large quantities when yeast are grown in mediums rich in glucose can be utilized to obtain anti-TNF peptides or Ab fragments of the present invention. Known glycolytic genes can also provide very efficient transcriptional control signals. For example, the promoter and terminator signals of the phosphoglycerate kinase gene can be utilized.

Production of anti-TNF peptides or Ab fragments or functional derivatives thereof in insects can be achieved, for example, by infecting the insect host with a baculovirus engineered to express transmembrane polypeptide by methods known to those of skill. See Ausubel et al, eds. *Current Protocols in Molecular Biology*, Wiley Interscience, §§16.8–16.11 (1987, 1993).

In a preferred embodiment, the introduced nucleotide sequence will be incorporated into a plasmid or viral vector capable of autonomous replication in the recipient host. Any

of a wide variety of vectors may be employed for this purpose. See, e.g., Ausubel et al, infra, §§ 1.5, 1.10, 7.1, 7.3, 8.1, 9.6, 9.7, 13.4, 16.2, 16.6, and 16.8–16.11. Factors of importance in selecting a particular plasmid or viral vector include: the ease with which recipient cells that contain the vector may be recognized and selected from those recipient cells which do not contain the vector; the number of copies of the vector which are desired in a particular host; and whether it is desirable to be able to "shuttle" the vector between host cells of different species.

Preferred prokaryotic vectors known in the art include plasmids such as those capable of replication in *E. coli* (such as, for example, pBR322, ColE1, pSC101, pACYC 184, πVX). Such plasmids are, for example, disclosed by Maniatis, T., et al. (*Molecular Cloning, A Laboratory Manual*, Second Edition, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (1989); Ausubel, infra. Bacillus plasmids include pC194, pC221, pT127, etc. Such plasmids are disclosed by Gryczan, T. (In: *The Molecular Biology of the Bacilli*, Academic Press, NY (1982), pp. 307–329). Suitable Streptomyces plasmids include pIJ101 (Kendall, K. J., et al., *J. Bacteriol.* 169:4177–4183 (1987)), and streptomyces bacteriophages such as φC31 (Chater, K. F., et al., In: *Sixth International Symposium on Actinomycetales Biology*, Akademiai Kaido, Budapest, Hungary (1986), pp. 45–54). Pseudomonas plasmids are reviewed by John, J. F., et al. (*Rev. Infect. Dis.* 8:693–704 (1986)), and Izaki, K. (*Jpn. J. Bacteriol.* 33:729–742 (1978); and Ausubel et al, supra).

Alternatively, gene expression elements useful for the expression of cDNA encoding anti-TNF antibodies or peptides include, but are not limited to (a) viral transcription promoters and their enhancer elements, such as the SV40 early promoter (Okayama, et al., *Mol. Cell. Biol.* 3:280 (1983)), Rous sarcoma virus LTR (Gorman, et al., *Proc. Natl. Acad. Sci., USA* 79:6777 (1982)), and Moloney murine leukemia virus LTR (Grosschedl, et al., *Cell* 41:885 (1985)); (b) splice regions and polyadenylation sites such as those derived from the SV40 late region (Okayama et al., infra); and (c) polyadenylation sites such as in SV40 (Okayama et al., infra).

Immunoglobulin cDNA genes can be expressed as described by Liu et al., infra, and Weidle et al., *Gene* 51:21 (1987), using as expression elements the SV40 early promoter and its enhancer, the mouse immunoglobulin H chain promoter enhancers, SV40 late region mRNA splicing, rabbit β-globin intervening sequence, immunoglobulin and rabbit β-globin polyadenylation sites, and SV40 polyadenylation elements. For immunoglobulin genes comprised of part cDNA, part genomic DNA (Whittle et al., *Protein Engineering* 1:499 (1987)), the transcriptional promoter is human cytomegalovirus, the promoter enhancers are cytomegalovirus and mouse/human immunoglobulin, and mRNA splicing and polyadenylation regions are from the native chromosomal immunoglobulin sequences.

In one embodiment, for expression of cDNA genes in rodent cells, the transcriptional promoter is a viral LTR sequence, the transcriptional promoter enhancers are either or both the mouse immunoglobulin heavy chain enhancer and the viral LTR enhancer, the splice region contains an intron of greater than 31 bp, and the polyadenylation and transcription termination regions are derived from the native chromosomal sequence corresponding to the immunoglobulin chain being synthesized. In other embodiments, cDNA sequences encoding other proteins are combined with the above-recited expression elements to achieve expression of the proteins in mammalian cells.

Each fused gene is assembled in, or inserted into, an expression vector. Recipient cells capable of expressing the chimeric immunoglobulin chain gene product are then transfected singly with an anti-TNF peptide or chimeric H or chimeric L chain-encoding gene, or are co-transfected with a chimeric H and a chimeric L chain gene. The transfected recipient cells are cultured under conditions that permit expression of the incorporated genes and the expressed immunoglobulin chains or intact antibodies or fragments are recovered from the culture.

In one embodiment, the fused genes encoding the anti-TNF peptide or chimeric H and L chains, or portions thereof, are assembled in separate expression vectors that are then used to co-transfect a recipient cell.

Each vector can contain two selectable genes, a first selectable gene designed for selection in a bacterial system and a second selectable gene designed for selection in a eukaryotic system, wherein each vector has a different pair of genes. This strategy results in vectors which first direct the production, and permit amplification, of the fused genes in a bacterial system. The genes so produced and amplified in a bacterial host are subsequently used to co-transfect a eukaryotic cell, and allow selection of a co-transfected cell carrying the desired transfected genes.

Examples of selectable genes for use in a bacterial system are the gene that confers resistance to ampicillin and the gene that confers resistance to chloramphenicol. Preferred selectable genes for use in eukaryotic transfectants include the xanthine guanine phosphoribosyl transferase gene (designated gpt) and the phosphotransferase gene from Tn5 (designated neo).

Selection of cells expressing gpt is based on the fact that the enzyme encoded by this gene utilizes xanthine as a substrate for purine nucleotide synthesis, whereas the analogous endogenous enzyme cannot. In a medium containing (1) mycophenolic acid, which blocks the conversion of inosine monophosphate to xanthine monophosphate, and (2) xanthine, only cells expressing the gpt gene can survive. The product of the neo blocks the inhibition of protein synthesis by the antibiotic G418 and other antibiotics of the neomycin class.

The two selection procedures can be used simultaneously or sequentially to select for the expression of immunoglobulin chain genes introduced on two different DNA vectors into a eukaryotic cell. It is not necessary to include different selectable markers for eukaryotic cells; an H and an L chain vector, each containing the same selectable marker can be co-transfected. After selection of the appropriately resistant cells, the majority of the clones will contain integrated copies of both H and L chain vectors and/or anti-TNF peptides.

Alternatively, the fused genes encoding the chimeric H and L chains can be assembled on the same expression vector.

For transfection of the expression vectors and production of the chimeric antibody, the preferred recipient cell line is a myeloma cell. Myeloma cells can synthesize, assemble and secrete immunoglobulins encoded by transfected immunoglobulin genes and possess the mechanism for glycosylation of the immunoglobulin. A particularly preferred recipient cell is the recombinant Ig-producing myeloma cell SP2/0 (ATCC #CRL 8287). SP2/0 cells produce only immunoglobulin encoded by the transfected genes. Myeloma cells can be grown in culture or in the peritoneal cavity of a mouse, where secreted immunoglobulin can be obtained from ascites fluid. Other suitable recipient cells include lymphoid cells such as B lymphocytes of human or non-human origin, hybridoma cells of human or non-human origin, or interspecies heterohybridoma cells.

The expression vector carrying a chimeric antibody construct or anti-TNF peptide of the present invention can be introduced into an appropriate host cell by any of a variety of suitable means, including such biochemical means as transformation, transfection, conjugation, protoplast fusion, calcium phosphate-precipitation, and application with polycations such as diethylaminoethyl (DEAE) dextran, and such mechanical means as electroporation, direct microinjection, and microprojectile bombardment (Johnston et al., *Science* 240:1538 (1988)). A preferred way of introducing DNA into lymphoid cells is by electroporation (Potter et al., *Proc. Natl. Acad. Sci. USA* 81:7161 (1984); Yoshikawa, et al.,*Jpn. J. Cancer Res.* 77:1122–1133). In this procedure, recipient cells are subjected to an electric pulse in the presence of the DNA to be incorporated. Typically, after transfection, cells are allowed to recover in complete medium for about 24 hours, and are then seeded in 96-well culture plates in the presence of the selective medium. G418 selection is performed using about 0.4 to 0.8 mg/ml G418. Mycophenolic acid selection utilizes about 6 μg/ml plus about 0.25 mg/ml xanthine. The electroporation technique is expected to yield transfection frequencies of about 10^{-5} to about 10^{-4} for Sp2/0 cells. In the protoplast fusion method, lysozyme is used to strip cell walls from catarrhal harboring the recombinant plasmid containing the chimeric antibody gene. The resulting spheroplasts are fused with myeloma cells with polyethylene glycol.

The immunoglobulin genes of the present invention can also be expressed in nonlymphoid mammalian cells or in other eukaryotic cells, such as yeast, or in prokaryotic cells, in particular bacteria.

Yeast provides substantial advantages over bacteria for the production of immunoglobulin H and L chains. Yeasts carry out post-translational peptide modifications including glycosylation. A number of recombinant DNA strategies now exist which utilize strong promoter sequences and high copy number plasmids which can be used for production of the desired proteins in yeast. Yeast recognizes leader sequences of cloned maamalian gene products and secretes peptides bearing leader sequences (i.e., pre-peptides) (Hitzman, et al., 11*th International Conference on Yeast, Genetics and Molecular Biology*, Montpelier, France, Sep. 13–17, 1982).

Yeast gene expression systems can be routinely evaluated for the levels of production, secretion and the stability of anti-TNF peptides, antibody and assembled murine and chimeric antibodies, fragments and regions thereof. Any of a series of yeast gene expression systems incorporating promoter and termination elements from the actively expressed genes coding for glycolytic enzymes produced in large quantities when yeasts are grown in media rich in glucose can be utilized. Known glycolytic genes can also provide very efficient transcription control signals. For example, the promoter and terminator signals of the phosphoglycerate kinase (PGK) gene can be utilized. A number of approaches can be taken for evaluating optimal expression plasmids for the expression of cloned immunoglobulin cDNAs in yeast (see Glover, ed., *DNA Cloning*, Vol. II, pp45–66, IRL Press, 1985).

Bacterial strains can also be utilized as hosts for the production of antibody molecules or peptides described by this invention, *E. coli* K12 strains such as *E. coli* W3110 (ATCC 27325), and other enterobacteria such as *Salmonella typhimurium* or *Serratia marcescens*, and various Pseudomonas species can be used.

Plasmid vectors containing replicon and control sequences which are derived from species compatible with a host cell are used in connection with these bacterial hosts. The vector carries a replication site, as well as specific genes which are capable of providing phenotypic selection in transformed cells. A number of approaches can be taken for evaluating the expression plasmids for the production of murine and chimeric antibodies, fragments and regions or antibody chains encoded by the cloned immunoglobulin cDNAs in bacteria (see Glover, ed., *DNA Cloning*, Vol. I, IRL Press, 1985, Ausubel, infra, Sambrook, infra, Colligan, infra).

Preferred hosts are mammalian cells, grown in vitro or in vivo. Mammalian cells provide post-translational modifications to immunoglobulin protein molecules including leader peptide removal, folding and assembly of H and L chains, glycosylation of the antibody molecules, and secretion of functional antibody protein.

Mammalian cells which can be useful as hosts for the production of antibody proteins, in addition to the cells of lymphoid origin described above, include cells of fibroblast origin, such as Vero (ATCC CRL 81) or CHO-K1 (ATCC CRL 61).

Many vector systems are available for the expression of cloned anti TNF peptides H and L chain genes in mammalian cells (see Glover, ed., *DNA Cloning*, Vol. II, pp143–238, IRL Press, 1985). Different approaches can be followed to obtain complete H_2L_2 antibodies. As discussed above, it is possible to co-express H and L chains in the same cells to achieve intracellular association and linkage of H and L chains into complete tetrameric H_2L_2 antibodies and/or anti-TNF peptides. The co-expression can occur by using either the same or different plasmids in the same host. Genes for both H and L chains and/or anti-TNF peptides can be placed into the same plasmid, which is then transfected into cells, thereby selecting directly for cells that express both chains. Alternatively, cells can be transfected first with a plasmid encoding one chain, for example the L chain, followed by transfection of the resulting cell line with an H chain plasmid containing a second selectable marker. Cell lines producing anti-TNF peptides and/or H_2L_2 molecules via either route could be transfected with plasmids encoding additional copies of peptides, H, L, or H plus L chains in conjunction with additional selectable markers to generate cell lines with enhanced properties, such as higher production of assembled H_2L_2 antibody molecules or enhanced stability of the transfected cell lines.

Anti-idiotype Abs. In addition to monoclonal or chimeric anti-TNF antibodies, the present invention is also directed to an anti-idiotypic (anti-Id) antibody specific for the anti-TNF antibody of the invention. An anti-Id antibody is an antibody which recognizes unique determinants generally associated with the antigen-binding region of another antibody. The antibody specific for TNF is termed the idiotypic or Id antibody. The anti-Id can be prepared by immunizing an animal of the same species and genetic type (e.g. mouse strain) as the source of the Id antibody with the Id antibody or the antigen-binding region thereof. The immunized animal will recognize and respond to the idiotypic determinants of the immunizing antibody and produce an anti-Id antibody. The anti-Id antibody can also be used as an "immunogen" to induce an immune response in yet another animal, producing a so-called anti-anti-Id antibody. The anti-anti-Id can be epitopically identical to the original antibody which induced the anti-Id. Thus, by using antibodies to the idiotypic determinants of a mAb, it is possible to identify other clones expressing antibodies of identical specificity.

Accordingly, mAbs generated against TNF according to the present invention can be used to induce anti-Id antibod-

ies in suitable animals, such as BALB/c mice. Spleen cells from such immunized mice can be used to produce anti-Id hybridomas secreting anti-Id mAbs. Further, the anti-Id mAbs can be coupled to a carrier such as keyhole limpet hemocyanin (KLH) and used to immunize additional BALB/c mice. Sera from these mice will contain anti-anti-Id antibodies that have the binding properties of the original mAb specific for a TNF epitope.

Screening Methods for determining tissue necrosis factor neutralizing and/or inhibiting activity are also provided in the present invention. In the context of the present invention, TNF neutralizing activity or TNF inhibiting activity refers to the ability of a TNF neutralizing compound to block at least one biological activity of TNF, such as preventing TNF from binding to a TNF receptor, blocking production of TNF by intracellular processing, such as transcription, translation or post-translational modification, expression on the cell surface, secretion or assembly of the bioactive trimer of TNF. Additionally, TNF neutralizing compounds can act by inducing regulation of metabolic pathways such as those involving the up or down regulation of TNF production. Alternatively TNF neutralizing compounds can modulate cellular sensitivity to TNF by decreasing such sensitivity. TNF neutralizing compounds can be selected from the group consisting of antibodies, or fragments or portions thereof, peptides, peptido mimetic compounds or organo mimetic compounds that neutralizes TNF activity in vitro, in situ or in vivo is considered a TNF neutralizing compound if used according to the present invention. Screening methods which can be used to determine TNF neutralizing activity of a TNF neutralizing compound can include in vitro or in vivo assays. Such in vitro assays can include a TNF cytotoxicity assay, such as a radioimmuno assay which determine a decrease in cell death by contact with TNF, such as chimpanzee or human TNF in isolated or recombinant form, wherein the concurrent presence of a TNF neutralizing compound reduces the degree or rate of cell death. The cell death can be determined using ID50 values which represent the concentration of a TNF neutralizing compound which decreases the cell death rate by 50%. For example, MAb's A2 and cA2 are found to have ID50 about 17 mg/ml +/−3 mg/ml, such as 14–20 mg/ml, or any range or value therein. Such a TNF cytotoxicity assay is presented in example II.

Alternatively or additionally, another in vitro assay which can be used to determine neutralizing activity of a TNF neutralizing compound is an assay which measures the neutralization of TNF induced procoagulant activity, such as presented in example XI.

Alternatively or additionally, TNF neutralizing activity of a TNF neutralizing compound can be measured by an assay for the neutralization of TNF induced IL-6 secretion, such as using cultured human umbilical vein endothelial cells (HUVEC), for example. Also presented in example 11.

Alternatively or additionally, in vivo testing of TNF neutralizing activity of TNF neutralizing compounds can be tested using survival of mouse given lethal doses of Rh TNF with controlled and varied concentrations of a TNF neutralizing compound, such as TNF antibodies. Preferably galactosamine sensitive mice are used. For example, using a chimeric human anti-TNF antibody as a TNF neutralizing compound, a concentration of 0.4 milligrams per kilogram TNF antibody resulted in a 70–100% increase in survival and a 2.0 mg/kg dose of TNF antibody resulted in a 90–100% increase in survival rate using such an assay, for example, as presented in example 12.

Additionally, after TNF neutralizing compounds are tested for safety in animal models such as chimpanzees, for example as presented in Example XVII, TNF neutralizing compounds can be used to treat various TNF related pathologies, as described herein, and as presented in Examples XVIII–XXII.

Accordingly, any suitable TNF neutralizing compound can be used in methods according to the present invention. Examples of such TNF neutralizing compound can be selected from the group consisting of antibodies or portions thereof specific to neutralizing epitopes of TNF, p55 receptors, p75 receptors, or complexes thereof, portions of TNF receptors which bind TNF, peptides which bind TNF, any peptido mimetic drugs which bind TNF and any organo mimetic drugs that block TNF.

Such TNF neutralizing compounds can be determined by routine experimentation based on the teachings and guidance presented herein, by those skilled in the relevant arts. Structural Analogs of Anti-TNF Antibodies and Anti-TNF Peptides

Structural analogs of anti-TNF Abs and peptides of the present invention are provided by known method steps based on the teaching and guidance presented herein.

Knowledge of the three-dimensional structures of proteins is crucial in understanding how they function. The three-dimensional structures of more than 400 proteins are currently available in protein structure databases (in contrast to around 15,000 known protein sequences in sequence databases). Analysis of these structures shows that they fall into recognizable classes of motifs. It is thus possible to model a three-dimensional structure of a protein based on the proteins homology to a related protein of known structure. Many examples are known where two proteins that have relatively low sequence homology, can have very similar three dimensional structures or motifs.

In recent years it has become possible to determine the three dimensional structures of proteins of up to about 15 kDa by nuclear magnetic resonance (NMR). The technique only requires a concentrated solution of pure protein. No crystals or isomorphous derivatives are needed. The structures of a number proteins have been determined by this method. The details of NMR structure determination are well-known in the art (See, e.g., Wuthrich, *NMR of Proteins and Nucleic Acids*, Wiley, New York, 1986; Wuthrich, K. *Science* 243:45–50 (1989); Clore et al., *Crit. Rev. Bioch. Molec. Biol.* 24:479–564 (1989); Cooke et al. *Bioassays* 8:52–56 (1988), which references are hereby incorporated herein by reference).

In applying this approach, a variety of ^{1}H NMR 2D data sets are collected for anti-TNF Abs and/or anti-TNF peptides of the present invention. These are of two main types. One type, COSY (Correlated Spectroscopy) identifies proton resonances that are linked by chemical bonds. These spectra provide information on protons that are linked by three or less covalent bonds. NOESY (nuclear Overhauser enhancement spectroscopy) identifies protons which are close in space (less than 0.5 nm). Following assignment of the complete spin system, the secondary structure is defined by NOESY. Cross peaks (nuclear Overhauser effects or NOE's) are found between residues that are adjacent in the primary sequence of the peptide and can be seen for protons less than 0.5 nm apart. The data gathered from sequential NOE's combined with amide proton coupling constants and NOE's from non-adjacent amino acids, that are adjacent to the secondary structure, are used to characterize the secondary structure of the polypeptides. Aside from predicting secondary structure, NOE's indicate the distance that protons are in space in both the primary amino acid sequence and the secondary structures. Tertiary structure predictions are determined, after all the data are considered, by a "best fit" extrapolation.

Types of amino acid are first identified using through-bond connectivities. The second step is to assign specific amino acids using through-space connectivities to neighboring residues, together with the known amino acid sequence. Structural information is then tabulated and is of three main kinds: The NOE identifies pairs of protons which are close in space, coupling constants give information on dihedral angles and slowly exchanging amide protons give information on the position of hydrogen bonds. The restraints are used to compute the structure using a distance geometry type of calculation followed by refinement using restrained molecular dynamics. The output of these computer programs is a family of structures which are compatible with the experimental data (i.e. the set of pairwise <0.5 nm distance restraints). The better that the structure is defined by the data, the better the family of structures can be superimposed, (i.e., the better the resolution of the structure). In the better defined structures using NMR, the position of much of backbone (i.e. the amide, Cα and carbonyl atoms) and the side chains of those amino acids that lie buried in the core of the molecule can be defined as clearly as in structures obtained by crystallography. The side chains of amino acid residues exposed on the surface are frequently less well defined, however. This probably reflects the fact that these surface residues are more mobile and can have no fixed position. (In a crystal structure this might be seen as diffuse electron density).

Thus, according to the present invention, use of NMR spectroscopic data is combined with computer modeling to arrive structural analogs of at least portions of anti-TNF Abs and peptides based on a structural understanding of the topography. Using this information, one of ordinary skill in the art will know how to achieve structural analogs of anti-TNF Abs and/or peptides, such as by rationally-based amino acid substitutions allowing the production of peptides in which the TNF binding affinity is modulated in accordance with the requirements of the expected therapeutic or diagnostic use of the molecule, preferably, the achievement of greater specificity for TNF binding.

Alternatively, compounds having the structural and chemical features suitable as anti-TNF therapeutics and diagnostics provide structural analogs with selective TNF affinity. Molecular modeling studies of TNF binding compounds, such as TNF receptors, anti-TNF antibodies, or other TNF binding molecules, using a program such as MACROMODEL®, INSIGHT®, and DISCOVER® provide such spatial requirements and orientation of the anti-TNF Abs and/or peptides according to the present invention. Such structural analogs of the present invention thus provide selective qualitative and quantitative anti-TNF activity in vitro, in situ and/or in vivo.

Therapeutic Methods for Treating TNF-Related Pathologies The anti-TNF peptides, antibodies, fragments and/or derivatives of the present invention are useful for treating a subject having a pathology or condition associated with abnormal levels of a substance reactive with an anti-TNF antibody, in particular TNF, such as TNFα or TNFβ, in excess of, or less than, levels present in a normal healthy subject, where such excess or diminished levels occur in a systemic, localized or particular tissue type or location in the body. Such tissue types can include, but are not limited to, blood, lymph, CNS, liver, kidney, spleen, heart muscle or blood vessels, brain or spinal cord white matter or grey matter, cartilage, ligaments, tendons, lung, pancreas, ovary, testes, prostrate. Increased or decreased TNF concentrations relative to normal levels can also be localized to specific regions or cells in the body, such as joints, nerve blood vessel junctions, bones, specific tendons or ligaments, or sites of infection, such as bacterial or viral infections.

TNF related pathologies include, but are not limited to, the following:

(A) acute and chronic immune and autoimmune pathologies, such as systemic lupus erythematosus (SLE) rheumatoid arthritis, thyroidosis, graft versus host disease, scleroderma, diabetes mellitus, Graves' disease, and the like;

(B) infections, including, but not limited to, sepsis syndrome, cachexia, circulatory collapse and shock resulting from acute or chronic bacterial infection, acute and chronic parasitic and/or infectious diseases, bacterial, viral or fungal, such as a HIV, AIDS (including symptoms of cachexia, autoimmune disorders, AIDS dementia complex and infections);

(C) inflammatory diseases, such as chronic inflammatory pathologies and vacsular inflammatory pathologies, including chronic inflammatory pathologies such as sarcoidosis, chronic inflammatory bowel disease, ulcerative colitis, and Crohn's pathology and vascular inflammatory pathologies, such as, but not limited to, disseminated intravascular coagulation, atherosclerosis, and Kawasaki's pathology;

(D) neurodegenerative diseases, including, but are not limited to,

demyelinating diseases, such as multiple sclerosis and acute transverse myelitis;

extrapyramidal and cerebellar disorders' such as lesions of the corticospinal system;

disorders of the basal ganglia or cerebellar disorders;

hyperkinetic movement disorders such as Huntington's Chorea and senile chorea;

drug-induced movement disorders, such as those induced by drugs which block CNS dopamine receptors;

hypokinetic movement disorders, such as Parkinson's disease;

Progressive supranucleo palsy;

Cerebellar and Spinocerebellar Disorders, such as astructural lesions of the cerebellum;

spinocerebellar degenerations (spinal ataxia, Friedreich's ataxia, cerebellar cortical degenerations, multiple systems degenerations (Mencel, Dejerine-Thomas, Shi-Drager, and Machado-Joseph); and systemic disorders (Refsum's disease, abetalipoprotemia, ataxia, telangiectasia, and mitochondrial multi.system disorder);

demyelinating core disorders, such as multiple sclerosis, acute transverse myelitis;

disorders of the motor unit, such as neurogenic muscular atrophies (anterior horn cell degeneration, such as amyotrophic lateral sclerosis, infantile spinal muscular atrophy and juvenile spinal muscular atrophy); Alzheimer's disease; Down's Syndrome in middle age; Diffuse Lewy body disease; Senile Dementia of Lewy body type; Wernicke-Korsakoff syndrome; chronic alcoholism; Creutzfeldt-Jakob disease; Subacute sclerosing panencephalitis, Hallerrorden-Spatz disease; and Dementia pugilistica, or any subset thereof;

(E) malignant pathologies involving TNF-secreting tumors or other malignancies involving TNF, such as, but not limited to leukemias (acute, chronic myelocytic, chronic lymphocytic and/or myelodyspastic syndrome); lymphomas (Hodgkin's and non-

Hodgkin's lymphomas, such as malignant lymphomas (Burkitt's lymphoma or Mycosis fungoides)); and

(F) alcohol-induced hepatitis.

See, e.g., Berkow et al, eds., The Merck Manual, 16th edition, chapter 11, pp 1380–1529, Merck and Co., Rahway, N.J., 1992, which reference, and references cited therein, are entirely incorporated herein by reference.

Such treatment comprises parenterally administering a single or multiple doses of the antibody, fragment or derivative. Preferred for human pharmaceutical use are high affinity potent hTNFα-inhibiting and/or neutralizing murine and chimeric antibodies, fragments and regions of this invention.

Anti-TNF peptides or MAbs of the present invention can be administered by any means that enables the active agent to reach the agent's site of action in the body of a mammal. In the case of the antibodies of this invention, the primary focus is the ability to reach and bind with TNF released by monocytes and macrophages or other TNF producing cells. Because proteins are subject to being digested when administered orally, parenteral administration, i.e., intravenous, subcutaneous, intramuscular, would ordinarily be used to optimize absorption.

Therapeutic Administration

Anti-TNF peptides and/or MAbs of the present invention can be administered either as individual therapeutic agents or in combination with other therapeutic agents. They can be administered alone, but are generally administered with a pharmaceutical carrier selected on the basis of the chosen route of administration and standard pharmaceutical practice.

The dosage administered will, of course, vary depending upon known factors such as the pharmacodynamic characteristics of the particular agent, and its mode and route of administration; age, health, and weight of the recipient; nature and extent of symptoms, kind of concurrent treatment, frequency of treatment, and the effect desired. Usually a daily dosage of active ingredient can be about 0.01 to 100 milligrams per kilogram of body weight. Ordinarily 1.0 to 5, and preferably 1 to 10 milligrams per kilogram per day given in divided doses 1 to 6 times a day or in sustained release form is effective to obtain desired results.

As a non-limiting example, treatment of TNF-related pathologies humans or animals can be provided as a daily dosage of anti-TNF peptides, monoclonal chimeric and/or murine antibodies of the present invention 0.1 to 100 mg/kg, such as 0.5, 0.9, 1.0, 1.1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 45, 50, 60, 70, 80, 90 or 100 mg/kg, per day, on at least one of day 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40, or alternatively, at least one of week 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20, or any combination thereof, using single or divided doses of every 24, 12, 8, 6, 4, or 2 hours, or any combination thereof.

Since circulating concentrations of TNF tend to be extremely low, in the range of about 10 pg/ml in non-septic individuals, and reaching about 50 pg/ml in septic patients and above 100 pg/ml in the sepsis syndrome (Hammerle, A. F. et al., 1989, infra) or can be only be detectable at sites of TNF-mediated pathology, it is preferred to use high affinity and/or potent in vivo TNF-inhibiting and/or neutralizing antibodies, fragments or regions thereof, for both TNF immunoassays and therapy of TNF-mediated pathology. Such antibodies, fragments, or regions, will preferably have an affinity for hTNFα, expressed as Ka, of at least 10^8 M^{-1}, more preferably, at least 10^9 M^{-1}, such as 10^8–10^{10} M^{-1}, 5×10^8 M^{-1}, 8×10^8 M^{-1}, 2×10^9 M^{-1}, 4×10^9 M^{-1}, 6×10^9 M^{-1}, 8×10^9 M^{-1}, or any range or value therein.

Preferred for human therapeutic use are high affinity murine and chimeric antibodies, and fragments, regions and derivatives having potent in vivo TNFα-inhibiting and/or neutralizing activity, according to the present invention, that block TNF-induced IL-6 secretion. Also preferred for human therapeutic uses are such high affinity murine and chimeric anti-TNFα antibodies, and fragments, regions and derivatives thereof, that block TNF-induced procoagulant activity, including blocking of TNF-induced expression of cell adhesion molecules such as ELAM-1 and ICAM-1 and blocking of TNF mitogenic activity, in vivo, in situ, and in vitro.

Dosage forms (composition) suitable for internal administration generally contain from about 0.1 milligram to about 500 milligrams of active ingredient per unit. In these pharmaceutical compositions the active ingredient will ordinarily be present in an amount of about 0.5–95% by weight based on the total weight of the composition.

For parenteral administration, anti-TNF peptides or antibodies can be formulated as a solution, suspension, emulsion or lyophilized powder in association with a pharmaceutically acceptable parenteral vehicle. Examples of such vehicles are water, saline, Ringer's solution, dextrose solution, and 5% human serum albumin. Liposomes and nonaqueous vehicles such as fixed oils can also be used. The vehicle or lyophilized powder can contain additives that maintain isotonicity (e.g., sodium chloride, mannitol) and chemical stability (e.g., buffers and preservatives). The formulation is sterilized by commonly used techniques.

Suitable pharmaceutical carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, A. Osol, a standard reference text in this field of art.

For example, a parenteral composition suitable for administration by injection is prepared by dissolving 1.5% by weight of active ingredient in 0.9% sodium chloride solution.

Anti-TNF peptides and/or antibodies of this invention can be adapted for therapeutic efficacy by virtue of their ability to mediate antibody-dependent cellular cytotoxicity (ADCC) and/or complement-dependent cytotoxicity (CDC) against cells having TNF associated with their surface. For these activities, either an endogenous source or an exogenous source of effector cells (for ADCC) or complement components (for CDC) can be utilized. The murine and chimeric antibodies, fragments and regions of this invention, their fragments, and derivatives can be used therapeutically as immunoconjugates (see for review: Dillman, R. O., Ann. Int. Med. 111:592–603 (1989)). Such peptides or Abs can be coupled to cytotoxic proteins, including, but not limited to ricin-A, Pseudomonas toxin and Diphtheria toxin. Toxins conjugated to antibodies or other ligands or peptides are well known in the art (see, for example, Olsnes, S. et al., *Immunol. Today* 10:291–295 (1989)). Plant and bacterial toxins typically kill cells by disrupting the protein synthetic machinery.

Anti-TNF peptides and/or antibodies of this invention can be conjugated to additional types of therapeutic moieties including, but not limited to, radionuclides, therapeutic agents, cytotoxic agents and drugs. Examples of radionuclides which can be coupled to antibodies and delivered in vivo to sites of antigen include ^{212}Bi, ^{131}I, ^{186}Re, and ^{90}Y, which list is not intended to be exhaustive. The radionuclides exert their cytotoxic effect by locally irradiating the cells, leading to various intracellular lesions, as is known in the art of radiotherapy.

Cytotoxic drugs which can be conjugated to anti-TNF peptides and/or antibodies and subsequently used for in vivo therapy include, but are not limited to, daunorubicin, doxorubicin, methotrexate, and Mitomycin C. Cytotoxic drugs interfere with critical cellular processes including DNA, RNA, and protein synthesis. For a description of these classes of drugs which are well known in the art, and their mechanisms of action, see Goodman, et al., *Goodman and Gilman's THE PHARMACOLOGICAL BASIS OF THERAPEUTICS*, 8th Ed., Macmillan Publishing Co., 1990.

Anti-TNF peptides and/or antibodies of this invention can be advantageously utilized in combination with other monoclonal or murine and chimeric antibodies, fragments and regions, or with lymphokines or hemopoietic growth factors, etc., which serve to increase the number or activity of effector cells which interact with the antibodies.

Anti-TNF peptides and/or antibodies, fragments or derivatives of this invention can also be used in combination with TNF therapy to block undesired side effects of TNF. Recent approaches to cancer therapy have included direct administration of TNF to cancer patients or immunotherapy of cancer patients with lymphokine activated killer (LAK) cells (Rosenberg et al., *New Eng. J. Med.* 313:1485–1492 (1985)) or tumor infiltrating lymphocytes (TIL) (Kurnick et al. (*Clin. Immunol. Immunopath.* 38:367–380 (1986); Kradin et al., *Cancer Immunol. Immunother.* 24:76–85 (1987); Kradin et al., *Transplant. Proc.* 20:336–338 (1988)). Trials are currently underway using modified LAK cells or TIL which have been transfected with the TNF gene to produce large amounts of TNF. Such therapeutic approaches are likely to be associated with a number of undesired side effects caused by the pleiotropic actions of TNF as described herein and known in the related arts. According to the present invention, these side effects can be reduced by concurrent treatment of a subject receiving TNF or cells producing large amounts of TIL with the antibodies, fragments or derivatives of the present invention. Effective doses are as described above. The dose level will require adjustment according to the dose of TNF or TNF-producing cells administered, in order to block side effects without blocking the main anti-tumor effect of TNF. One of ordinary skill in the art will know how to determine such doses without undue experimentation.

Treatment of Arthritis. In rheumatoid arthritis, the main presenting symptoms are pain, stiffness, swelling, and loss of function (Bennett J C. The etiology of rheumatoid arthritis. In Textbook of Rheumatology (Kelley W N, Harris E D, Ruddy S, Sledge C B, eds.) W B Saunders, Philadelphia pp 879–886, 1985). The multitude of drugs used in controlling such symptoms seems largely to reflect the fact that none is ideal. Although there have been many years of intense research into the biochemical, genetic, microbiological, and immunological aspects of rheumatoid arthritis, its pathogenesis is not completely understood, and none of the treatments clearly stop progression of joint destruction (Harris E D. Rheumatoid Arthritis: The clinical spectrum. In Textbook of Rheumatology (Kelley, et al., eds.) W B Saunders, Philadelphia pp 915–990, 1985).

TNFα is of major importance in the pathogenesis of rheumatoid arthritis. TNFα is present in rheumatoid arthritis joint tissues and synovial fluid at the protein and mRNA level (Buchan G, Barrett K, Turner M, Chantry D, Maini R N, and Feldmann M. Interleukin-1 and tumour necrosis factor mRNA expression in rheumatoid arthritis: prolonged production of IL-1_{α}. *Clin. Exp. Immunol* 73: 449–455, 1988), indicating local synthesis. However detecting TNFα in rheumatoid arthritis joints even in quantities sufficient for bioactivation does not necessarily indicate that it is important in the pathogenesis of rheumatoid arthritis, nor that it is a good candidate therapeutic target. In order to address these questions, the effects of anti-TNF antibody and peptides (rabbit or monoclonal) on rheumatoid joint cell cultures, and for comparison, osteoarthritic cell cultures, have been studied. IL-1 production was abolished, showing TNFα as a suitable therapeutic target for the therapy of rheumatoid arthritis, since anti-TNFα blocks both TNF and IL-1, the two cytokines known to be involved in cartilage and bone destruction (Brennan et al., *Lancet* 11: 244–247, 1989).

Subsequent studies in rheumatoid arthritis tissues have supported this hypothesis. Anti-TNF Abs abrogated the production of another proinflammatory cytokine, GM-CSF (Haworth et al., *Eur. J. Immunol.* 21:2575–2579, 1991). This observation has been independently confirmed (Alvaro-Gracia et al., 1991). It has also been demonstrated that anti-TNF diminishes cell adhesion and HLA class II expression in rheumatoid arthritis joint cell cultures.

Diagnostic Methods

The present invention also provides the above anti-TNF peptides and antibodies, detectably labeled, as described below, for use in diagnostic methods for detecting TNFα in patients known to be or suspected of having a TNFα-mediated condition.

Anti-TNF peptides and/or antibodies of the present invention are useful for immunoassays which detect or quantitate TNF, or anti-TNF antibodies, in a sample. An immunoassay for TNF typically comprises incubating a biological sample in the presence of a detectably labeled high affinity anti-TNF peptide and/or antibody of the present invention capable of selectively binding to TNF, and detecting the labeled peptide or antibody which is bound in a sample. Various clinical assay procedures are well known in the art, e.g., as described in *Immunoassays for the 80's*, A. Voller et al., eds., University Park, 1981.

Thus, an anti-TNF peptide or antibody, can be added to nitrocellulose, or other solid support which is capable of immobilizing cells, cell particles or soluble proteins. The support can then be washed with suitable buffers followed by treatment with the detectably labeled TNF-specific peptide or antibody. The solid phase support can then be washed with the buffer a second time to remove unbound peptide or antibody. The amount of bound label on the solid support can then be detected by known method steps.

By "solid phase support" or "carrier" is intended any support capable of binding peptide, antigen or antibody. Well-known supports or carriers, include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the carrier can be either soluble to some extent or insoluble for the purposes of the present invention. The support material can have virtually any possible structural configuration so long as the coupled molecule is capable of binding to TNF or an anti-TNF antibody. Thus, the support configuration can be spherical, as in a bead, or cylindrical, as in the inside surface of a test tube, or the external surface of a rod. Alternatively, the surface can be flat such as a sheet, culture dish, test strip, etc. Preferred supports include polystyrene beads. Those skilled in the art will know many other suitable carriers for binding antibody, peptide or antigen, or can ascertain the same by routine experimentation.

Well known method steps can determine binding activity of a given lot of anti-TNF peptide and/or antibody. Those skilled in the art can determine operative and optimal assay conditions by routine experimentation.

Detectably labeling a TNF-specific peptide and/or antibody can be accomplished by linking to an enzyme for use in an enzyme immunoassay (EIA), or enzyme-linked immunosorbent assay (ELISA). The linked enzyme reacts with the exposed substrate to generate a chemical moiety which can be detected, for example, by spectrophotometric, fluorometric or by visual means. Enzymes which can be used to detectably label the TNF-specific antibodies of the present invention include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholinesterase.

By radioactively labeling the TNF-specific anti-bodies, it is possible to detect TNF through the use of a radioimmunoassay (RIA) (see, for example, Work, et al., *Laboratory Techniques and Biochemistry in Molecular Biology*, North Holland Publishing Company, N.Y. (1978). The radio-active isotope can be detected by such means as the use of a gamma counter or a scintillation counter or by autoradiography. Isotopes which are particularly useful for the purpose of the present invention are: ^{3}H, ^{125}I, ^{131}I, ^{35}S, ^{14}C, and, preferably, ^{125}I.

It is also possible to label the TNF-specific antibodies with a fluorescent compound. When the fluorescent labeled antibody is exposed to light of the proper wave length, its presence can then be detected due to fluorescence. Among the most commonly used fluorescent labelling compounds are fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine.

The TNF-specific antibodies can also be detectably labeled using fluorescence-emitting metals such as ^{152}Eu, or others of the lanthanide series. These metals can be attached to the TNF-specific antibody using such metal chelating groups as diethylenetriaminepentaacetic acid (DTPA) or ethylenediamine-tetraacetic acid (EDTA).

The TNF-specific antibodies also can be detectably labeled by coupling to a chemiluminescent compound. The presence of the chemiluminescently labeled antibody is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of particularly useful chemiluminescent labeling compounds are luminol, isoluminol, theromatic acridinium ester, imidazole, acridinium salt and oxalate ester.

Likewise, a bioluminescent compound can be used to label the TNF-specific antibody, fragment or derivative of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Important bioluminescent compounds for purposes of labeling are luciferin, luciferase and aequorin.

Detection of the TNF-specific antibody, fragment or derivative can be accomplished by a scintillation counter, for example, if the detectable label is a radioactive gamma emitter, or by a fluorometer, for example, if the label is a fluorescent material. In the case of an enzyme label, the detection can be accomplished by colorometric methods which employ a substrate for the enzyme. Detection can also be accomplished by visual comparison of the extent of enzymatic reaction of a substrate in comparison with similarly prepared standards.

For the purposes of the present invention, the TNF which is detected by the above assays can be present in a biological sample. Any sample containing TNF can be used. Preferably, the sample is a biological fluid such as, for example, blood, serum, lymph, urine, inflammatory exudate, cerebrospinal fluid, amniotic fluid, a tissue extract or homogenate, and the like. However, the invention is not limited to assays using only these samples, it being possible for one of ordinary skill in the art to determine suitable conditions which allow the use of other samples.

In situ detection can be accomplished by removing a histological specimen from a patient, and providing the combination of labeled antibodies of the present invention to such a specimen. The antibody (or fragment) is preferably provided by applying or by overlaying the labeled antibody (or fragment) to a biological sample. Through the use of such a procedure, it is possible to determine not only the presence of TNF but also the distribution of TNF in the examined tissue. Using the present invention, those of ordinary skill will readily perceive that any of a wide variety of histological methods (such as staining procedures) can be modified in order to achieve such in situ detection.

The antibody, fragment or derivative of the present invention can be adapted for utilization in an immunometric assay, also known as a "two-site" or "sandwich" assay. In a typical immunometric assay, a quantity of unlabeled antibody (or fragment of antibody) is bound to a solid support that is insoluble in the fluid being tested and a quantity of detectably labeled soluble antibody is added to permit detection and/or quantitation of the ternary complex formed between solid-phase antibody, antigen, and labeled antibody.

Typical, and preferred, immunometric assays include "forward" assays in which the antibody bound to the solid phase is first contacted with the sample being tested to extract the TNF from the sample by formation of a binary solid phase antibody-TNF complex. After a suitable incubation period, the solid support is washed to remove the residue of the fluid sample, including unreacted TNF, if any, and then contacted with the solution containing a known quantity of labeled antibody (which functions as a "reporter molecule"). After a second incubation period to permit the labeled antibody to complex with the TNF bound to the solid support through the unlabeled antibody, the solid support is washed a second time to remove the unreacted labeled antibody. This type of forward sandwich assay can be a simple "yes/no" assay to determine whether TNF is present or can be made quantitative by comparing the measure of labeled antibody with that obtained for a standard sample containing known quantities of TNF. Such "two-site" or "sandwich" assays are described by Wide (*Radioimmune Assay Method*, Kirkham, ed., Livingstone, Edinburgh, 1970, pp. 199–206).

Other type of "sandwich" assays, which can also be useful with TNF, are the so-called "simultaneous" and "reverse" assays. A simultaneous assay involves a single incubation step wherein the antibody bound to the solid support and labeled antibody are both added to the sample being tested at the same time. After the incubation is completed, the solid support is washed to remove the residue of fluid sample and uncomplexed labeled antibody. The presence of labeled antibody associated with the solid support is then determined as it would be in a conventional "forward" sandwich assay.

In the "reverse" assay, stepwise addition first of a solution of labeled antibody to the fluid sample followed by the addition of unlabeled antibody bound to a solid support after a suitable incubation period, is utilized. After a second

incubation, the solid phase is washed in conventional fashion to free it of the residue of the sample being tested and the solution of unreacted labeled antibody. The determination of labeled antibody associated with a solid support is then determined as in the "simultaneous" and "forward" assays. In one embodiment, a combination of antibodies of the present invention specific for separate epitopes can be used to construct a sensitive three-site immunoradiometric assay.

TNF REMOVAL FROM SOLUTIONS

The murine and chimeric antibodies, fragments and regions, fragments, or derivatives of this invention, attached to a solid support, can be used to remove TNF from fluids or tissue or cell extracts. In a preferred embodiment, they are used to remove TNF from blood or blood plasma products. In another preferred embodiment, the murine and chimeric antibodies, fragments and regions are advantageously used in extracorporeal immunoadsorbent devices, which are known in the art (see, for example, *Seminars in Hematology*, 26 (2 Suppl. 1) (1989)). Patient blood or other body fluid is exposed to the attached antibody, resulting in partial or complete removal of circulating TNF (free or in immune complexes), following which the fluid is returned to the body. This immunoadsorption can be implemented in a continuous flow arrangement, with or without interposing a cell centrifugation step. See, for example, Terman, et al., *J. Immunol.* 117:1971–1975 (1976).

Having now generally described the invention, the same will be further understood by reference to certain specific examples which are included herein for purposes of illustration only and are not intended to be limiting unless otherwise specified.

EXAMPLE I

Production a Mouse Anti-Human TNF mAb

To facilitate clinical study of TNF mAb a high-affinity potent inhibiting and/or neutralizing mouse anti-human TNF IgG1 mAb designated A2 was produced.

Female BALB/c mice, 10 weeks old, were obtained from the Jackson Laboratory (Bar Harbor, Me.). Forty μg of purified *E. coli*-derived recombinant human TNF (rhTNF) emulsified with an equal volume of complete Freund's adjuvant (obtained from Difco Laboratories) in 0.4 ml was injected subcutaneously and intraperitoneally (i.p.) into a mouse. One week later, an injection of 5 μg of rhTNF in incomplete Freund's adjuvant was given i.p. followed by four consecutive i.p. injections of 10 μg of TNF without adjuvant. Eight weeks after the last injection, the mouse was boosted i.p. with 10 μg of TNF.

Four days later, the mouse was sacrificed, the spleen was obtained and a spleen cell suspension was prepared. Spleen cells were fused with cells of the nonsecreting hybridoma, Sp2/0 (ATCC CRL1581), at a 4:1 ratio of spleen cells to Sp2/0 cells, in the presence of 0.3 ml of 30% polyethylene glycol, PEG 1450. After incubation at 37° C. for 6 hours, the fused cells were distributed in 0.2 ml aliquots into 96-well plates at concentrations of 2×10^4 SP2/0 cells per well. Feeder cells, in the form of 5×10^4 normal BALB/c spleen cells, were added to each well.

The growth medium used consisted of RPMI-1640 medium, 10% heat-inactivated fetal bovine serum (FBS) (HYCLONE), 0.1 mM minimum essential medium (MEM) nonessential amino acids, 1 mM sodium pyruvate, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin (GIBCO Laboratories) and, for selection, hypoxanthine-aminopterin-thymidine (HAT) (Boehringer Mannheim). A solid-phase radioimmunoassay (RIA) was employed for screening supernatants for the presence of mAbs specific for rhTNFα. This assay is described in Example II, below. The background binding in this assay was about 500 cpm. A supernatant was considered positive if it yielded binding of 2000 cpm or higher.

Of 322 supernatants screened, 25 were positive by RIA. Of these 25, the one with the highest binding (4800 cpm) was designated A2. Positive wells were subcloned at limiting dilution on mouse feeder cells. Upon further analysis of the supernatants in neutralization assays, A2 was found to be the only positive clone showing potent inhibiting and/or neutralizing activity. Thus, the hybridoma line A2 was selected. This line was maintained in RPMI-1640 medium with 10% FBS (GIBCO), 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin.

Alternatively, anti-TNF antibodies which inhibit TNF biological activity can be screened by binding to peptide including at least 5 amino acids of residues 87–108 or both residues 59–80 and 87–108 of TNF (of SEQ ID NO:1) or combinations of peptides contained therein, which are used in place of the rTNF protein, as described above.

EXAMPLE II

Characterization of an Anti-TNF Antibody of the Present Invention

Radioimmunoassays

E. coli-derived rhTNF was diluted to 1 μg/ml in BCB buffer, pH 9.6, and 0.1 ml of the solution was added to each assay well. After incubation at 4° C. overnight, the wells were washed briefly with BCB, then sealed with 1% bovine incubated with 40 pg/ml of natural (GENZYME, Boston, Mass.) or recombinant (SUNTORY, Osaka, Japan) human TNFα with varying concentrations of mAb A2 in the presence of 20 μg/ml cycloheximide at 39° C. overnight. Controls included medium alone or medium +TNF in each well. Cell death was measured by staining with naphthol blue-black, and the results read spectrophotometrically at 630 nm. Absorbance at this wave length correlates with the number of live cells present.

It was found that A2 inhibited or neutralized the cytotoxic effect of both natural and rhTNF in a dose-dependent manner (FIG. **3**).

In another experiment, the specificity of this inhibiting and/or neutralizing activity was tested. A673/6 cells were seeded at 3×10^4 cells/well 20 hr before the TNF bioassay. Two-fold serial dilutions of rhTNF, *E. coli*-derived recombinant human lymphotoxin (TNFβ), and *E. coli*-derived recombinant murine TNF were prepared. The A2 hybridoma supernatant was added to an equal volume of the diluted TNF preparations, and the mixtures were incubated at room temperature for 30 min. Aliquots of 0.1 ml were transferred to the wells containing A673/6 cells, 20 μg/ml of cycloheximide was added, and the cells were incubated at 39° C. overnight. The cells were then fixed and stained for evaluation of cytotoxicity. The results indicate that mAb A2 specifically inhibited or neutralized the cytotoxicity of rhTNFα, whereas it had no effect on human lymphotoxin (TNFβ) (FIG. **4**) or murine TNF (FIG. **5**).

Experiments were next performed to analyze the cross-reactivity of mAb A2 with TNF derived from non-human primates. Monocytes isolated from B514 (baboon), J91 (cynomolgus) and RH383 (rhesus) blood by Ficoll gradient centrifugation and adherence, were incubated at 1×10^5 cells/well in RPMI 1640 medium with 5% FBS and 2 μg/ml of *E.*

coli LPS for 3 or 16 hr at 37° C. to induce TNF production. Supernatants from duplicate wells were pooled and stored at 4° C. for less than 20 hr until the TNF bioassay was performed, as described above, using A673/6 cells. Two-fold dilutions of the culture supernatants were mixed with either medium or purified mAb A2 at a final concentration of 1 μg/ml, incubated at room temperature for 30 min and aliquots transferred to the indicator cells. The results showed that mAb A2 failed to significantly inhibit or neutralize the cytotoxic activity of TNF produced by baboon, cynomolgus and rhesus monkey monocytes.

A further experiment was conducted with chimpanzee TNF. Monocytes isolated from CH563 (chimpanzee) blood were incubated as described above to generate TNF-containing supernatants. The ability of 10 μg/ml of mAb A2 to inhibit or neutralize the bioactivity of these supernatants was assayed as above. Human TNF was used as a positive control. Results, shown in FIG. **6**, indicate that mAb A2 had potent inhibiting and/or neutralizing activity for chimpanzee TNF, similar to that for human TNF (FIG. **7**).

The inhibiting and/or neutralizing activity of mAb A2 was compared with three other murine mAbs specific for human TNF, termed TNF-1, TNF-2 and TNF-3, and a control mAb. Two-fold serial dilutions of purified mAbs were mixed with rhTNF (40 pg/ml), incubated at room temperature for 30 min, and aliquots tested for TNF bioactivity as above. It was found that mAbs TNF-1, TNF-2 and TNF-3 each had a similar moderate degree of inhibiting and/or neutralizing activity. In contrast, mAb A2 had much more potent inhibiting and/or neutralizing activity.

EXAMPLE III

General Strategy for Cloning Antibody V and C Genes

The strategy for cloning the V regions for the H and L chain genes from the hybridoma A2, which secretes the anti-TNF antibody described above, was based upon the linkage in the genome between the V region and the corresponding J (joining) region for functionally rearranged (and expressed) Ig genes. J region DNA probes can be used to screen genomic libraries to isolate DNA linked to the J regions. Although DNA in the germline configuration (i.e., unrearranged) would also hybridize to J probes, this DNA would not be linked to a Ig V region sequence and can be identified by restriction enzyme analysis of the isolated clones.

The cloning utilized herein was to isolate V regions from rearranged H and L chain genes using J_H and J_k probes. These clones were tested to see if their sequences were expressed in the A2 hybridoma by Northern analysis. Those clones that contained expressed sequence were cloned into expression vectors containing human C regions and transfected into mouse myeloma cells to determine if an antibody was produced. The antibody from producing cells was then tested for binding specificity and functionally compared to the A2 murine antibody.

EXAMPLE IV

Construction of a L Chain Genomic Library

To isolate the L chain V region gene from the A2 hybridoma, a size-selected genomic library was constructed using the phage lambda vector charon 27. High molecular weight DNA was isolated from A2 hybridoma cells and digested to completion with restriction endonuclease HindIII. The DNA was then fractionated on a 0.8% agarose gel and the DNA fragments of three different size ranges of approximately 3 kb, 4 kb and 6 kb were isolated from the gel by electroelution. The size ranges for library construction were chosen based upon the size of Hind III fragments that hybridized on a southern blot with the J_k probe. After phenol/chloroform extraction and ethanol precipitation, the DNA fragments from each size class were ligated with lambda charon 27 arms and packaged into phage particles in vitro using Gigapack Gold from Stratagene (LaJolla, Calif.).

These libraries were screened directly at a density of approximately 20,000 plaques per 150 mm petri dish using a ^{32}P-labeled J_k probe. The mouse L chain J_k probe was a 2.7 kb HindIII fragment containing all five J_k segments. The probe was labeled with ^{32}P by random priming using a kit obtained from Boehringer Mannheim. Free nucleotides were removed by centrifugation through a Sephadex G-50 column. The specific activities of the probe was approximately 10^9 cpm/μg.

Plaque hybridizations were carried out in 5×SSC, 50% formamide, 2×Denhardt's reagent, and 200 μg/ml denatured salmon sperm DNA at 42° C. for 18–20 hours. Final washes were in 0.5×SSC, 0.1% SDS at 65° C. Positive clones were identified after autoradiography.

EXAMPLE V

Construction of H Chain Genomic Library

To isolate the V region gene for the A2 H chain, a genomic library was constructed in the lambda gt10 vector system. High molecular weight DNA was digested to completion with restriction endonuclease EcoRI and fragments of approximately 7.5 kb were isolated after agarose gel electrophoresis. These fragments were ligated with lambda gt10 arms and packaged into phage particles in vitro using Gigapack Gold.

This library was screened at a density of 20,000 plaques per 150 mm plate using a J_H probe. The J_H probe was a 2 kb BamHI/EcoRI fragment containing both J3 and J4 segments. The probe was labeled as in Example III and had a similar specific radioactivity. Hybridization and wash conditions were identical to those used in Example III.

EXAMPLE VI

Cloning of the TNF-Specific V Gene Regions

Several positive clones were isolated from the H and L chain libraries after screening approximately 10^6 plaques from each library using the J_H and J_k probes, respectively. Following plaque purification, bacteriophage DNA was isolated for each positive clone, digested with either EcoRI (H chain clones) or HindIII (L chain clones) and fractionated on 1% agarose gels. The DNA was transferred to nitrocellulose and the blots were hybridized with the J_H or the J_K probe.

Several H chain clones were obtained that contained 7.5 kb EcoRI DNA encoding fragments of MAbs to the J_H probe. For the light chain libraries, several clones from each of the three size-selected libraries were isolated that contained HindIII fragments that hybridize to the J_k probe. For the L chain, several independently derived HindIII fragments of 2.9 kb from the 2 kb library hybridized with a 1250 bp mRNA from A2, but not with SP2/0 mRNA (see Example VII). In addition, several HindIII fragments derived from the 4 kb library hybridized both to the A2 mRNA and the fusion partner mRNA. A 5.7 kb HindIII fragment from the 6 kb library did not hybridize to either RNA.

The observed lengths of hybridizing A2 mRNA were the correct sizes for H and L chain mRNA, respectively. Because the RNA expression was restricted to the A2 hybridoma, it was assumed that the 7.5 kb H chain fragments and the 2.9 kb L chain fragments contained the correct V region sequences from A2. One example of each type was chosen for further study. The important functional test is the demonstration that these V regions sequences, when combined with appropriate C region sequences, are capable of directing the synthesis of an antibody with a specificity and affinity similar to that of the murine A2 antibody.

The 7.5 kb H chain fragment and the 2.9 kb L chain fragment were subcloned into plasmid vectors that allow expression of the chimeric mouse/human proteins in murine myeloma cells (see Examples VIII and IX). These plasmids were co-transfected into SP2/0 cells to ascertain if intact antibody molecules were secreted, and if so, if they were of the correct specificity and affinity. Control transfections were also performed pairing the putative anti-TNF H chain with an irrelevant, but expressed, L chain; the putative anti-TNF L chain was also paired with an irrelevant, but expressed, H chain. The results indicated that the 7.5 kb H chain fragment could be expressed, whereas the 2.9 kb L chain fragment could not. This was confirmed by DNA sequence analysis that suggested portions of the coding region were not in the proper amino acid reading frame when compared to other known L chain amino acid sequences.

Because the 2.9 kb HindIII fragment appeared not to contain a functional V gene, the 4.0 kb and 5.7 kb HindIII fragments isolated from L chain libraries were cloned into expression vectors and tested for expression of chimeric antibody after co-transfection with the 7.5 kb H chain. The 5.7 kb HindIII fragment was incapable of supporting antibody expression, whereas the 4.0 kb HindIII fragment did support antibody expression. The antibody resulting from the co-transfection of the 7.5 kb putative H chain V region and the 4.0 kb L chain V region was purified, tested in solid phase TNF binding assay, and found to be inactive. It was concluded that the V region contained on the 4.0 kb HindIII fragment was not the correct anti-TNF V regions, but was contributed to the hybridoma by the fusion partner. This was subsequently confirmed by sequence analysis of cDNA derived from the A2 hybridoma and from the fusion partner.

Other independently derived L chain clones containing 2.9 kb HindIII fragments that hybridized with A2 mRNA were characterized in more detail. Although the restriction maps were similar, the clones fell into two classes with respect to the presence or absence of an AccI enzyme site. The original (non-functional) 2.9 kb fragment (designated clone 8.3) was missing an AccI site present in some other clones (represented by clone 4.3). The DNA sequence of clone 4.3 was extremely similar to clone 8.3, but contained a single amino acid reading frame with close homology to known L chains, unlike clone 8.3. The 2.9 kb HindIII fragment from clone 4.3 was subcloned into the L chain expression vector and co-transfected with the putative anti-TNF H chain into SP2/0 cells. An antibody was synthesized, purified and tested in the solid phase TNF binding assay. This antibody bound to TNF, and therefore, the clone 4.3 L chain V region was assumed to be the correct one.

The A2 murine hybridoma has been shown to contain at least four rearranged L chain V region genes. At least two of these are expressed as proteins: clone 4.3 (the correct anti-TNF L chain gene) and the gene contained in the 4.0 kb HindIII fragment (contributed by the fusion partner). The expression of two L chains implies that the resulting antibody secreted from the murine hybridoma is actually a mixture of antibodies, some using the correct L chain, some using the incorrect L chain, and some using one of each. The presence of two different L chains in the murine A2 antibody has been confirmed by SDS gel and N-terminal protein sequence analysis of the purified antibody. Because construction of the chimeric A2 antibody involves cloning the individual H and L chain genes and expressing them in a non-producing cell line, the resulting antibody will have only the correct L chain and therefore should be a more potent antibody (see Examples X, XI and XII).

EXAMPLE VII

Northern Analysis of Cloned DNA

Cloned DNA corresponding to the authentic H and L chain V regions from the A2 hybridoma would be expected to hybridize to A2 mRNA. Non-functional DNA rearrangements at either the H or L chain genetic loci should not be expressed.

Ten μg total cellular RNA was subjected to electrophoresis on 1% agarose/formaldehyde gels (Sambrook et al, infra) and transferred to nitrocellulose. Blots were hybridized with random primed DNA probes in 50% formamide, 2×Denhardt's solution, 5×SSC, and 200 μg/ml denatured salmon sperm DNA at 42° C. for 10 hours. Final wash conditions were 0.5×SSC, 0.1% SDS at 65° C.

The subdoned DNA fragment s were labeled with ^{32}P by random priming and hybridized to Northern blots containing total RNA derived from A2 cells or from cells of SP2/0, the fusion partner parent of A2. The 7.5 kb EcoRI H chain fragment hybridized with a 2 kb mRNA from A2, but not with SP2/0 mRNA. Similarly, the 2.9 kb L chain HindIII fragment (clone 4.3) hybridized with a 1250 bp mRNA from A2, but not with SP2/0 mRNA. The observed lengths of A2 mRNA hybridizing were the correct sizes for H and L chain mRNA, respectively, confirming that the V region sequences on these DNA fragments are expressed in A2 hybridoma cells.

EXAMPLE VIII

Construction of Expression Vectors

The putative L (clone 4.3) and H chain V genes described above were joined to human kappa and gaimal constant region genes in expression vecters. The 7.5 kb EcoRI fragment corresponding to the putative V_H region gene from A2 was cloned into an expression vector containing the human C_{gamma1} gene and the Ecogpt gene to yield the plasmid designated pA2HG1apgpt (see FIG. **8**).

The 2.9 kb putative V_L fragment from clone 4.3 was cloned into a vector containing the human kappa C_k gene and the Ecogpt gene to allow selection in mammalian cells. The resulting plasmid was designated pA2HuKapgpt (See FIG. **8**).

EXAMPLE IX

Expression of Chimeric Antibody Genes

To express the chimeric H and L chain genes, the expression plasmids were transfected into cells of the non-producing mouse myeloma cell line, SP2/0. Plasmid DNA to be transfected was purified by centrifuging to equilibrium in ethidium bromide/cesium chloride gradients twice. Plasmid DNA (10–50 μg) was added to 10^7 SP2/0 cells in medium containing Hank's salts, and the mixture was placed in a

BIORAD electroporation apparatus. Electroporation was performed at 20 volts, following which the cells were plated in 96 well microtiter plates.

Mycophenolic acid selection was applied after 24 hours and drug resistant colonies were identified after 1–2 weeks. Resistant colonies were expanded to stable cell lines and tissue culture supernatant from these cell lines was tested for antibody using an ELISA assay with goat anti-human IgG Fc antibody and goat anti-human H+L conjugated with alkaline phosphatase (obtained from Jackson Laboratories).

The chimeric A2 antibody was purified from tissue culture supernatant by Protein A-Sepharose chromatography. The supernatant was adjusted to 0.1M Tris, 0.002M EDTA, pH 8.0 and loaded on a Protein A-Sepharose column equilibrated in the same buffer. The IgG was eluted with 0.1M citrate, pH 3.5, inhibited or neutralized with 1M Tris, and dialyzed into phosphate buffered saline (PBS).

The purified chimeric antibody was evaluated for its binding and inhibiting and/or neutralizing activity.

EXAMPLE X

Specificity of an Anti-TNF Chimeric Antibody

Since the antigen binding domain of cA2 was derived from murine A2, these mAbs would be expected to compete for the same binding site on TNF. Fixed concentrations of chimeric A2 and murine mAb A2 were incubated with increasing concentrations of murine and chimeric A2 competitor, respectively, in a 96-well microtiter plate coated with rhTNF (Dainippon, Osaka, Japan). Alkaline-phosphatase conjugated anti-human immunoglobulin and anti-mouse immunoglobulin second antibodies were used to detect the level of binding of chimeric and murine A2, respectively. Cross-competition for TNF antigen was observed in this solid-phase ELISA format (FIG. **9**). This finding is consistent with the expected identical epitope specificity of cA2 and murine A2.

The affinity constant for binding of mouse mAb A2 and cA2 to rhTNFα was determined by Scatchard analysis (see, for example, Scatchard, *Ann. N.Y. Acad. Sci.* 51:660 (1949)). The results are shown in FIG. **10**. This analysis involved measuring the direct binding of ^{125}I labelled cA2 to immobilized rhTNFα in a 96-well plate. The antibodies were each labelled to a specific activity of about 9.7 μCi/μg by the iodogen method. An affinity constant (Ka) of 0.5×10^9 liters/mole was calculated for the mouse mAb A2. Unexpectedly, the chimeric A2 antibody had a higher affinity, with a Ka of 1.8×10^9 liters/mole. Thus, the chimeric anti-TNFα antibody of the present invention was shown to exhibit a significantly higher affinity of binding to human TNFα than did the parental murine A2 mAb. This finding was surprising, since murine and chimeric antibodies, fragments and regions would be expected to have affinities that are equal to or less than that of the parent mAb.

Such high affinity anti-TNF antibodies, having affinities of binding to TNFα of at least 1×10^8 M^{-1}, more preferably at least 1×10^9 M^{-1} (expressed as Ka) are preferred for immunoassays which detect very low levels of TNF in biological fluids. In addition, anti-TNF antibodies having such high affinities are preferred for therapy of TNF-α-mediated conditions or pathology states.

The specificity of cA2 for TNF was confirmed by testing for cross-neutralization of human lymphotoxin (TNF-β). Lymphotoxin shares some sequence homology and certain biological activities, for example, tumor cell cytotoxicity, with TNF (Pennica, et al., *Nature* 312:724–729 (1984)). Cultured human A673 cells were incubated with increasing concentrations of human lymphotoxin (GENENTECH, San Francisco, Calif.) with or without 4 μg/ml chimeric A2 in the presence of 20 μg/ml cycloheximide at 39° C. overnight. Cell death was measured by vital staining with naphthol blue-black, as above. The results indicated that cA2 was ineffective at inhibiting and/or neutralizing human lymphotoxin, confirming the TNFα-specificity of the chimeric antibody.

The ability of A2 or cA2 to react with TNF from different animal species was also evaluated. As mentioned earlier, there are multiple epitopes on human TNF to which inhibiting and/or neutralizing mAbs will bind (Moller, et al., infra). Human TNF has bioactivity in a wide range of host animal species. However, certain inhibiting and/or neutralizing epitopes on human TNF are conserved amongst different animal species and others appear to be restricted to humans and chimpanzees.

Neutralization experiments utilized endotoxin-activated cell supernatants from freshly isolated human, chimpanzee, rhesus and cynomolgus monkey, baboon, pig, dog, rabbit, or rat monocytes as the TNF source. As discussed above, murine mAb A2 inhibited or neutralized activity of only human and chimpanzee TNF, and had no effect on TNF derived from other primates and lower animals. A2 also did not inhibit or neutralize the cytotoxic effect of recombinant mouse TNF.

Thus, the epitope recognized by A2 is one shared by human and chimpanzee TNFα. Chimeric A2 was also tested in this manner for cross-reactivity with monocyte-derived TNF from rat, rabbit, dog and pig, as well as with purified recombinant mouse TNFα, and natural and recombinant human TNFα. Chimeric A2 only inhibited or neutralized natural and recombinant human TNFα. Therefore, cA2 appears to share species specificity with murine A2.

EXAMPLE XI

In Vitro Activity and Neutralization Efficacy of a Chimeric Anti-TNF Antibody

Both the murine and chimeric anti-TNFα antibodies, A2 and cA2 were determined to have potent TNF-inhibiting and/or neutralizing activity. In the TNF cytotoxicity assay described above, murine A2, at a concentration of about 125 ng/ml completely inhibited or neutralized the biological activity of a 40 pg/ml challenge of rhTNFα. Two separate determinations of inhibiting and/or neutralizing potency, expressed as the 50% Inhibitory Dose (ID50) were determined to be 15.9±1.01 and 17.9±1.6 ng/ml (Mean±Std error). Thus the mAb A2 has an ID50 of about 17 ng/ml.

In this same experimental system, three other murine anti-TNFα antibodies (termed TNF-1, TNF-2 and TNF-3) of comparable binding affinity to TNF were found to have ID50 values of 1–2 orders of magnitude greater, and thus were significantly less potent in neutralization than A2.

The ability of cA2 to inhibit or neutralize human TNFα bioactivity in vitro was tested using the bioassay system described above. Cultured A673 cells were incubated with 40 pg/ml natural (Genzyme, Boston, Mass.) or recombinant (Suntory, Osaka, Japan) human TNF with or without antibody overnight as above, and cell death was measured by vital staining. As expected based upon the above results with the A2 mouse mAb, cA2 also inhibited or neutralized both natural and rhTNF in a dose-dependent manner in the cytotoxicity assay (FIG. **11**). In this assay format, levels of cA2 as low as 125 ng/ml completely abolished the toxic

activity of TNF. Upon repeated analysis, the cA2 exhibited greater TNF-inhibiting and/or neutralizing activity than did the parent murine A2 mAb. Such inhibiting and/or neutralizing potency, at antibody levels below 1 μg/ml, can easily be attained in the blood of a subject to whom the antibody is administered. Accordingly, such highly potent inhibiting and/or neutralizing anti-TNF antibodies, in particular the chimeric antibody, are preferred for therapeutic use in TNFα-mediated pathologies or conditions.

As mentioned above, TNF induces cellular secretion of IL-6. Furthermore, there is evidence that IL-6 is involved in the pathophysiology of sepsis, although the precise role of IL-6 in that syndrome is unclear (Fong, et al., *J Exp Med* 170:1627–1633 (1989); Starnes Jr., et al., *J Immunol* 145:4185–4191 (1990)). The ability of cA2 to inhibit or neutralize TNF-induced IL-6 secretion was evaluated using cultured human diploid FS-4 fibroblasts. The results in Table 2 show that cA2 was effective in blocking IL-6 secretion in cells that had been incubated overnight with TNF. TNF-induced IL-6 secretion was not inhibited in the absence of a mAb or in the presence of a control mAb specific for an irrelevant antigen.

TABLE 2

IN VITRO NEUTRALIZATION OF TNF-INDUCED IL-6 SECRETRION

	TNF Concentration (ng/ml)			
Antibody	0	0.3	1.5	7.5
None	<0.20	1.36	2.00	2.56
Control mAb	<0.20	1.60	1.96	2.16
cA2	<0.20	<9.20	<0.20	0.30

Values represent mean concentrations of IL-6 of duplicate wells, in ng/ml. RhTNF (Suntory, Osaka, Japan), with or without 4 μg/ml antibody, was added to cultures of FS-4 fibroblasts and after 18 h, the supernatant was assayed for IL-6 using the QUANTIKINE Human IL-6 Immunoassay (from R&D Systems, Minneapolis, MN). Control mAb = chimeric mouse/human IgG1 anti-platelet mAb (7E3).

The ability of TNF to activate procoagulant and adhesion molecule activities of endothelial cells (EC) is thought to be an important component of pathology pathophysiology. In particular, this can be associated with the vascular damage, disseminated intravascular coagulation, and severe hypotension that is associated with the sepsis syndrome. Therefore, the ability of cA2 to block TNF-induced activation of cultured human umbilical vein endothelial cells (HUVEC) was evaluated. TNF stimulation of procoagulant activity was determined by exposing intact cultured HUVEC cells to TNF (with or without antibody) for 4 hours and analyzing a cell lysate in a human plasma clotting assay. The results in Table 3 show the expected upregulation by TNF of HUVEC procoagulant activity (reflected by a decreased clotting time). Chimeric antibody cA2 effectively inhibited or neutralized this TNF activity in a dose-dependent manner.

TABLE 3

IN VITRO NEUTRALIZATION OF TNF-INDUCED PROCOAGULANT ACTIVITY

		TNF Concentration (ng/ml)		
Antibody	μg/ml	250	25	0
None	—	64 ± 4*	63 ± 1	133 ± 13
Control Ab	10.00	74 ± 6	N.D.	178 ± 55

TABLE 3-continued

IN VITRO NEUTRALIZATION OF TNF-INDUCED PROCOAGULANT ACTIVITY

		TNF Concentration (ng/ml)		
Antibody	μg/ml	250	25	0
cA2	10.00	114 ± 5	185 ± 61	141 ± 18
cA2	3.30	113 ± 2	147 ± 3	N.D.
cA2	1.10	106 ± 1	145 ± 8	N.D.
A2	0.37	73 ± 17	153 ± 4	N.D.
cA2	0.12	64 ± 1	118 ± 13	N.D.

* Values represent mean plasma clotting time, in seconds (± S.D.). Clotting time was determined in normal human plasma after addition of the rhTNF (Dainippon, Osaka, Japan) with or without antibody-treated HUVEC lysate and Ca^{++} at 37° C. N.D. = Not done. Control Ab is a chimeric mouse/human IgG1 anti-CD4 antibody.

In addition to stimulating procoagulant activity, TNF also induces surface expression of endothelial cell adhesion molecules such as ELAM-1 and ICAM-1. The ability of cA2 to inhibit or neutralize this activity of TNF was measured using an ELAM-1 specific detection radioimmunoassay. Cultured HUVEC were stimulated with 250 ng/ml rhTNF (Dainippon, Osaka, Japan) with or without antibody at 37° C. overnight in a 96-well plate format. Surface expression of ELAM-1 was determined by sequential addition of a mouse anti-human ELAM-1 mAb and ^{125}I-labelled rabbit anti-mouse immunoglobulin second antibody directly to culture plates at 4° C.

As shown in FIG. **12**, TNF induced the expression of ELAM-1 on the surface of cultured HUVEC cells, and this activity was again effectively blocked in a dose-related manner by cA2.

Finally, TNF is known to stimulate mitogenic activity in cultured fibroblasts. Chimeric A2 inhibited or neutralized TNF-induced mitogenesis of human diploid FS-4 fibroblasts cultures, confirming the potent inhibiting and/or neutralizing capability of cA2 against a broad spectrum of in vitro TNF biological activities.

EXAMPLE XII

Determination of Amino Acid Sequences (epitope) on Human TNF-α Recognized by cA2 mAb

Reagents The following reagents are readily available from commercial sources. FMOC-L-Ala-OPfp, FMOC-L-Cys(Trt)-OPfp, FMOC-L-Asp(OtBu)-OPfp, FMOC-L-Glu (OtBu)-OPfp, FMOC-L-Phe-OPfp, FMOC-Gly-OPfp, FMOC-L-His(Boc)-OPfp, FMOC-L-Ile-OPfp, FMOC-L-Lys(Boc)-OPfp, FMOC-L-Leu-OPfp, FMOC-L-Asn-OPfp, FMOC-L-Pro-OPfp, FMOC-L-Gln-OPfp, FMOC-L-Arg (Mtr)-OPfp, FMOC-L-Ser(tBu)-ODhbt, FMOC-L-Thr (tBu)-ODhbt, FMOC-L-Val-OPfp, FMOC-L-Trp-OPfp, FMOC-L-Try(tBu)-OPfp, and 1-hydroxybenotriazol (HOBT) were obtained from Cambridge Research Biochemicals. Piperidine and was obtained from Applied Biosystems, Inc. 1-Methyl-2-Pyrrolidinone (NMP) was obtained from EM Science; Methanol from J T Baker; Acetic Anhydride from Applied Biosystems, Inc., Trifluoroaccetic acid (TFA) from Applied Biosystems, Inc.; Diisopropylamne (DIEA), Triethylamine, Dithiothreitol (DTT) and Anisole from Aldrich and Hydrochloric Acid (HCl) from J T Baker.

Abbreviations: FMOC, 9-fluorenylmethoxycarbonyl; tBu t-butyl ether; OtB, t-butyl ester; Boc, t-butyloxycarbonyl; Mtr, 4-methoxy-2,3,6-trimethylbenzenesulfonyl; Trt, trityl; OPfp, pentafluorophenylester; ODhbt. oxo-benzotriazone ster;

A chimeric antibody of the present invention, designated cA2, was used to determine which portions of the TNF amino acid sequence were involved in inhibitory binding by the antibody by epitope mapping, whereby the amino acid sequences of TNF-α recognized by cA2 have been identified.

The complete primary sequence of human TNFα, according to Pennica et al, Nature 312:724–729 (1984) is shown in FIG. **13** (SEQ ID NO:1). Overlapping decapeptides beginning with every second amino acid and covering the entire amino acid sequence of human TNF-α were synthesized on polyethylene pins using the method of Gysen (Gysen et al., Peptides: Chemistry and Biological, Proceedings of the Twelfth American Peptide Symposium, p. 519–523, Ed, G. R. Marshall, Escom, Leiden, 1988). Sets of peptide pins bearing free N-terminal amino groups and acetylated N-terminal amino groups were individually prepared. Both sets of peptide pins were incubated in solutions containing the anti-TNF mAb cA2 to determine the amino acid sequences that make up the cA2 epitope on human TNF-α, as described below. FIG. **14**A shows the results of binding to the overlapping decapeptides that comprise the entire sequence of human TNFα. The O.D. (optional density) correlates directly with the increased degree of cA2 binding. FIG. **14**B shows the results of binding of cA2 to the same set of peptide pins in the presence of human TNFα. This competitive binding study delineates peptides which can show non-specific binding to cA2.

There are at least two non-contiguous peptide sequences of TNF-α recognized by cA2. Using the conventional protein numbering system wherein the N-terminal amino acid is number 1, the cA2 mAb recognizes an epitope composed at least in part of amino acids located within residues 87–108 or both residues 59–80 and 87–108 of TNF (SEQ ID NO:1). FIG. **15** presents these non-contiguous sequences within the TNF sequence.

Unexpectedly, the mAb cA2 blocks the action of TNF-α without binding to the putative receptor binding locus, which can include one or more of, e.g., 11–13, 37–42, 49–57 or 155–157 of hTNFα (of SEQ ID NO:1). Preferred anti-TNF mAbs are those that inhibit this binding of human TNF-α to its receptors by virtue of their ability to bind to one or more of these peptide sequences. These antibodies can block the activity of TNF by virtue of binding to the cA2 epitope, such binding demonstrated to inhibit TNF activity. The identification of those peptide sequences recognized by cA2 provides the information necessary to generate additional MAbs with binding characteristics and therapeutic utility that parallel the embodiments of this application.

Peptide Pin Synthesis. Using an epitope mapping kit purchased from Cambridge Research Biochemicals, Inc. (CRB), dodecapeptides corresponding to the entire sequence of human TNF-α were synthesized on polyethylene pins.

A synthesis schedule was generated using the CRB epitope mapping software. Prior to the first amino acid coupling, the pins were deprotected with a 20% piperidine in NMP solution for 30 minutes at room temperature. After deprotected, the pins were washed with NMP for five minutes at room temperature, followed by three methanol washes. Following the wash steps, the pins were allowed to air dry for at least 10 minutes.

The following procedure was performed for each coupling cycle:

1) The amino acid derivatives and the HOBT were weighted out according to the weights required in the synthesis schedule.
2) The HOBT was dissolved in the appropriate amount of NMP according to the synthesis schedule.
3) The amino acid derivatives were dissolved in the recommended amount of HOBT solution and 150 microliters were pipeted into the appropriate wells as directed by the well position sheet of the synthesis schedule.
4) The blocks containing the pins were placed into the wells, and the "sandwich" units stored in plastic bags in a 30° C. water bath for 18 hours.
5) The pins were removed from the wells and washed once (for 5 minutes) with NMP, three times (for two minutes) with methanol and air dried for 10 minutes.
6) The pins were deprotected as described above and the procedure repeated.

To acetylate the peptides on one block of pins, the peptide pins were washed, deprotected and treated with 150 microliters of a solution containing NMP; acetic anhydride:triethylamine (5:2:1) for 90 minutes at 30° C., followed by the washing procedure outlined above. The second set of peptide pins was deprotected by not acetylated to give free N-terminal amino groups.

The final deprotection of the peptides to remove the side chain protecting groups was done using a mixture of TFA:anisole:dithiothreitol, 95:2.5:2.5 (v/v/w) for four hours at ambient temperature. After deprotection, the pins were air dried for 10 minutes, followed by a 15 minute sonication in a solution of 0.1% HCl in methanol/distilled water (1:1). The pins dried over night and were then ready for testing. ELISA Assay for cA2 Binding to TNF-α Peptide PINs

Reagents: Disruption Buffer: Sodium dihydrogen phosphate (31.2 g, Sigma cat # S-0751 or equivalent) and sodium dodecylsulfate (20.0 g, Sigma cat # L-3771 or equivalent) were dissolved in 2.0 L of milliQ water. The pH was adjusted to 7.2±0.1 with 50% w/w sodium hydroxide (VWR cat # VW6730-3 or equivalent).

Blocking Buffer: Sodium dihydrogen phosphate (0.39 g, Sigma cat #S-0751 or equivalent) disodium hydrogen phosphate (1.07 g, Baker cat # 3828-1 or equivalent) and sodium chloride (8.50 g, Baker cat # 3624-5 or equivalent were dissolved in 1.0 L of milliQ water. The pH was adjusted to 7.2±0.1 with 50% w/w sodium hydroxide (VWR cat VW6730-3 or equivalent). Chicken egg albumin (10.0 g, Sigma cat #A-5503 or equivalent) and bovine serum albumin (10.0 g, Sigma, cat #A-3294 or equivalent) were dissolved at room temperature with gentle stirring. The solution was filtered, and to the solution was added Tween 20 (2.0 ml, Sigma cat #P-13.79 or equivalent). The solution was stirred gently at room temperature for 30 min, filtered and stored at 40°.

PBS/Tween 20: A 10×concentrate was prepared by dissolving sodium dihydrogen phosphate (3.90 g, Sigma cat # S-0751 or equivalent), disodium hydrogen phosphate (10.70 g, Baker cat #3828-1 or equivalent) and sodium chloride (85.0 g, Baker cat #3624-5 or equivalent) in 1.0 L of milliQ water. The pH was adjusted to 7.2±0.1 with 50% w/w sodium hydroxide (VWR cat #VW 6730 or equivalent). To the solution was added Tween 20 (5.0 mL, Sigma cat #P-1379 or equivalent), and the mixture stirred gently. Just prior to use 100 mL of this solution was diluted to 1.0 L with milliQ water.

Substrate solution: Substrate buffer was prepared by dissolving citric acid (4.20 g, Malinckrodt cat #0627 or equivalent) and disodium hydrogen phosphate (7.10 g, Baker cat #3828-1 or equivalent) in 1.0 L of milliQ water. The pH was adjusted to 5.00 with 50% w/w sodium hydroxide (VWR cat #VW6730-3 or equivalent). Immediately

prior to use an OPD substrate tablet (30 mg, Sigma cat #P-8412 or equivalent and 30% (v/v) hydrogen peroxide (40 μL, Sigma cat #P-1379 or equivalent) were added to the substrate buffer 25.0 mL). The solution was wrapped in foil and mixed thoroughly.

4 NH_2SO_4; Sulfuric acid (53 mL, EM Science cat #SX1244-5 or equivalent) was slowly added to MILLI-Q water (447 mL) and cooled to room temperature prior to use.

Equipment: Molecular Devices Model nu-max plate reader or equivalent. Scientific Products Model R4140 Oscillating table shaker and equivalent. BRANSON Model 5200 ultra-sonic bath or equivalent. FINNPIPETTE Model 4172317 multichannel pipeter or equivalent. CORNING Model 25801 96 well disposable polystyrene Elisa Plates.

Prior to use and after each subsequent use the peptide pins were cleaned using the following procedure. Disruption buffer (2.0 L) was heated to 60° and placed in an ultra-sonic bath in a fume hood. To the disruption buffer was added dithiolthreitol (2.5 g, Sigma cat #D-0632 or equivalent). The peptide pins were sonicated in this medium for 30 min, washed thoroughly with milliQ waster, suspended in a boiling ethanol bath for 2 min, and air-dried.

Blocking buffer (200 μL) was added to a 96 well disposable polystyrene Elisa plate and the peptide pins suspended in the wells. The peptide pins and plate were incubated for 2 h at room temperature on an oscillating table shaker. The plates and peptide pins were washed with PBS/Tween 20 (four times). To each well was added a 20 μg/ml concentration of cA2 antibody (diluted with blocking buffer, 175 μL/well). TNF competition was done by incubation of TNFα (40 μg/ml) and cA2 (20 μg/ml) in BSA/ovalbumin/BBS for three hours at room temperature. The peptide pins were suspended in the plate and incubated at 4° overnight. The peptide pins and plate were washed with PBS/Tween 20 (four times). To each well was added anti-human goat antibody conjugated to horseradish peroxidase (diluted with blocking buffer to 1/2000, 175 μL/well, Jackson IMMUNORESEARCH Labs). The peptide pins were suspended in the plate, and incubated for 1 h at room temperature on a oscillating table shaker. The plates and peptide pins were washed with PBS/Tween 20 (four times). To each well added freshly prepared substrate solution (150 μL/well), the peptide pins were suspended in the plate and incubated for 1 h at room temperature on an oscillating table shaker. The peptide pins were removed and to each well is added 4N H_2SO_4 (50 μL). The plates were read in a Molecular Devices plate reader (490 nm, subtracting 650 nm as a blank), and the results are shown in FIGS. **14**A and **14**B, as described above.

EXAMPLE XIII

Production Mouse Anti-Human TNF mAb Using TNF Peptide Fragments

Female BALB/c mice, as in Example I above, are injected subcutaneously and intraperitoneally (i.p.) with forty μg of purified *E. coli*-derived recombinant human TNF (rhTNF) fragments comprising anti-TNF epitopes of at least 5 amino acids located within the non-contiguous sequence 59–80, 87–108 or both residues 59–80 and 87–108 of TNF (of SEQ ID NO:1), as presented above, emulsified with an equal volume of complete Freund's adjuvant (obtained from Difco Laboratories) in 0.4 ml is into a mouse. One week later, a booster injection of 5 μg of these rhTNF fragments in incomplete Freund's adjuvant is given i.p. followed by four consecutive i.p. injections of 10 μg of TNF fragments including anti-TNF epitopes including amino acids from residues 59–80, 87–108 or both 59–80 and 87–108 of hTNFα (of SEQ ID NO:1) without adjuvant. Eight weeks after the last injection, the mouse is boosted i.p. with 10 μg of TNF.

Four days later, the mouse is sacrificed, the spleen is obtained and a spleen cell suspension is prepared. Spleen cells are fused with cells of the nonsecreting hybridoma, Sp2/0 (ATCC CRL1581), at a 4:1 ratio of spleen cells to Sp2/0 cells, in the presence of 0.3 ml of 30% polyethylene glycol, PEG 1450. After incubation at 37° C. for 6 hours, the fused cells are distributed in 0.2 ml aliquots into 96-well plates at concentrations of 2×10^4 SP2/0 cells per well. Feeder cells, in the form of 5×10^4 normal BALB/c spleen cells, are added to each well.

The growth medium used consisted of RPMI-1640 medium, 10% heat-inactivated fetal bovine serum (FBS) (Hyclone), 0.1 mM MEM nonessential amino acids, 1 mM sodium pyruvate, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin (GIBCO Laboratories) and, for selection, hypoxanthine-aminopterin-thymidine (HAT) (Boehringer Mannheim). A solid-phase radioimmunoassay (RIA) is employed for screening supernatants for the presence of mabs specific for rhTNFα fragments including portions of residues 59–80, 87–108 or both 59–80 and 87–108 of hTNFα (of SEQ ID NO:1). This assay is described in Example II, above. The background binding in this assay is about 500 cpm. A supernatant is considered positive if it yielded binding of 2000 cpm or higher.

Of the supernatants screened, one or more positive supernatants are routinely identified by RIA. Of these positive supernatants, the highest binding (as shown by the higher cpm values) are subcloned at limiting dilution on mouse feeder cells. Upon further analysis of the supernatants in neutralization assays, routinely one or more antibodies are found to have potent inhibiting and/or neutralizing activity. These positive and inhibiting and/or neutralizing hybridoma-lines are then selected and maintained in RPMI-1640 medium with 10% FBS (GIBCO), 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin.

EXAMPLE XIV

Production of Murine and Chimeric Antibodies, Fragments and Regions from TNF Peptides

Murine and chimeric antibodies, fragments and regions are obtained by construction of chimeric expression vectors encoding the mouse variable region of antibodies obtained in Example XIII and human constant regions, as presented in Examples IV–IX above.

The resulting chimeric A2 antibody is purified from tissue culture supernatant by Protein A-Sepharose chromatography. The supernatant is adjusted to 0.1M Tris, 0.002M EDTA, pH 8.0 and loaded on a Protein A-Sepharose column equilibrated in the same buffer. The IgG is then eluted with 0.1M citrate, pH 3.5, neutralized with 1M Tris, and dialyzed into phosphate buffered saline (PBS).

The purified murine and chimeric antibodies, fragments and regions are evaluated for its binding and inhibiting and/or neutralizing activity.

EXAMPLE XV

In Vitro Activity and Neutralization Efficacy of a Chimeric Anti-TNF Antibody

Both the murine and chimeric anti-TNFα antibodies of the present invention, as obtained according to Examples

XIII and XIV, are determined to have potent TNF-inhibiting and/or neutralizing activity, as shown for example, in the TNF cytotoxicity assay described above, expressed as the 50% Inhibitory Dose (ID50).

In this same experimental system, three other murine anti-TNFα antibodies (termed TNF-1, TNF-2 and TNF-3) of comparable binding affinity to TNF are found to have ID50 values of 1–2 orders of magnitude greater, and thus have significantly less potent in neutralization, than both the murine and chimeric anti-TNFα antibodies of the present invention.

The ability of both the murine and chimeric anti-TNFα antibodies of the present invention, as obtained according to Examples XIII and XIV, to inhibit or neutralize human TNFα bioactivity in vitro is tested using the bioassay system described above. Cultured cells producing the murine or chimeric anti-TNFα antibodies of the present invention, as obtained according to Examples XIII and XIV, are incubated with 40 pg/ml natural (Genzyme, Boston, Mass.) or recombinant (Suntory, Osaka, Japan) human TNF with or without antibody overnight as above, and cell death is measured by vital staining. As expected, both the murine and chimeric anti-TNFα antibodies of the present invention, as obtained according to Examples XIII and XIV, inhibited or neutralized both natural and rhTNF in a dose-dependent manner in the cytotoxicity assay. Such inhibiting and/or neutralizing potency, at antibody levels below 1 μg/ml, can easily be attained in the blood of a subject to whom the antibody is administered. Accordingly, such highly potent inhibiting and/or neutralizing anti-TNF antibodies, in particular the chimeric antibody, are preferred for therapeutic use in TNFα-mediated pathologies or conditions.

The ability of cA2 to inhibit or neutralize TNF-induced IL-6 secretion is evaluated using cultured human diploid FS-4 fibroblasts. The results are expected to show that both murine and chimeric anti-TNFα antibodies of the present invention, as obtained according to Examples XIII and XIV, are effective in blocking IL-6 secretion in cells that had been incubated overnight with TNF. TNF-induced IL-6 secretion is not inhibited in the absence of a mAb or in the presence of a control mAb specific for an irrelevant antigen.

The ability of TNF to activate procoagulant and adhesion molecule activities of endothelial cells (EC) is thought to be an important component of pathology pathophysiology. In particular, this can be associated with the vascular damage, disseminated intravascular coagulation, and severe hypotension that is associated with the sepsis syndrome. Therefore, the ability of both the murine and chimeric anti-TNFα antibodies of the present invention, as obtained according to Examples XIII and XIV, to block TNF-induced activation of cultured human umbilical vein endothelial cells (HUVEC) is evaluated. TNF stimulation of procoagulant activity is determined by exposing intact cultured HUVEC cells to TNF (with or without antibody) for 4 hours and analyzing a cell lysate in a human plasma clotting assay. The results are expected to show the expected upregulation by TNF of HUVEC procoagulant activity (reflected by a decreased clotting time). Both the murine and chimeric anti-TNFα antibodies of the present invention, as obtained according to Examples XIII and XIV, are expected to effectively inhibit or neutralize this TNF activity in a dose-dependent manner.

In addition to stimulating procoagulant activity, TNF also induces surface expression of endothelial cell adhesion molecules such as ELAM-1 and ICAM-1. The ability of both the murine and chimeric anti-TNFα antibodies of the present invention, as obtained according to Examples XIII and XIV, are expected to inhibit or neutralize this activity of TNF is measured using an ELAM-1 specific detection radioimmunoassay. Cultured HUVEC are stimulated with 250 ng/ml rhTNF (Dainippon, Osaka, Japan) with or without antibody at 37° C. overnight in a 96-well plate format. Surface expression of ELAM-1 is determined by sequential addition of a mouse anti-human ELAM-1 mAb and ^{125}I-labelled rabbit anti-mouse immunoglobulin second antibody directly to culture plates at 4° C.

TNF is expected to induce the expression of ELAM-1 on the surface of cultured HUVEC cells, and this activity is again expected to be effectively blocked in a dose-related manner by both the murine and chimeric anti-TNFα antibodies of the present invention, as obtained according to Examples XIII and XIV.

Finally, TNF is known to stimulate mitogenic activity in cultured fibroblasts. Both the murine and chimeric anti-TNFα antibodies of the present invention, as obtained according to Examples XIII and XIV, are expected to inhibit or neutralize TNF-induced mitogenesis of human diploid FS-4 fibroblasts cultures, confirming the potent inhibiting and/or neutralizing capability of both the murine and chimeric anti-TNFα antibodies of the present invention, as obtained according to Examples XIII and XIV against a broad spectrum of in vitro TNF biological activities.

EXAMPLE XVI

In Vivo Activity and Efficacy of cA2 Antibody

Evidence that the potent in vitro inhibiting and/or neutralizing capability of cA2 is manifest in vivo was obtained. Earlier animal studies showed that administration of TNF to experimental animals mimics the pathology state obtained with either Gram-negative bacterial infection or direct endotoxin administration (Tracey, et al., 1986. infra; Tracey, et al., 1987, infra; Lehmann, et al., infra).

An in vivo model wherein lethal doses of human TNF are administered to galactosamine-sensitized mice (Lehmann, V. et al., infra) is substantially modified for testing the capability of both the murine and chimeric anti-TNFα antibodies of the present invention, as obtained according to Examples XIII and XIV above, to inhibit or neutralize TNF in vivo. An i.p. challenge with 5 μg (0.25 mg/kg) of rhTNF resulted in 80–90 percent mortality in untreated control animals and in animals treated i.v. 15–30 minutes later with either saline or 2 mg/kg control antibody (a chimeric IgG1 derived from murine 7E3 anti-platelet mAb). In contrast, treatment with both the murine and chimeric anti-TNFα antibodies of the present invention, as obtained according to Examples XIII and XIV, is expected to reduce mortality to 0–30 percent with 0.4 mg/kg of antibody, and to 0–10 percent with 20 mg/kgs. These expected results indicate that both the murine and chimeric anti-TNFα antibodies of the present invention, as obtained according to Examples XIII and XIV, are capable of inhibiting and/or neutralizing the biological activity of TNF in vivo as well as in vitro.

TABLE 4

PREVENTION OF HUMAN TNF-INDUCED LETHALITY BY CHIMERIC A2

Anitbody	Outcome (Survivors/Total) Experiment #1	Experiment #2
None	1/10	N.D.
Control Ab, 2 mg/kg	2/10	1/10
cA2 (2 mg/kg) (p = 0.0001)	9/10 (p = 0.0055)	10/10
cA2 (0.4 mg/kg) (p = 0.0001)	7/10 (p = 0.07)	10/10

Female C3H/HeN mice were administered 5 μg rhTNF (Dainippon, Osaka, Japan) + 18 mg galactosamine i.p. and antibody was administered 15–30 minutes later i.v. Deaths were recorded 48 h post-challenge. Control MAb = chimeric mouse/human IgG1 anti-platelet MAb (7E3). N.D. = not done. p values refer to comparison with the control Ab.

EXAMPLE XVII

cA2 MAb Safety in Chimpanzees

The epitope specificity of A2 can be for an epitope which predominates in humans and chimpanzees. Therefore, the chimpanzee was chosen as a relevant mammalian species to determine the toxicological potential and provide safety information for cA2. Chimpanzees were dosed at levels of 15 mg/kg for four to five consecutive days and 30 mg/kg once or for three consecutive days. No adverse clinical signs, and no changes considered to be cA2 treatment related were observed in the monitored parameters including routine hematology and blood chemistry. Thus, doses of up to 30 mg/kg for three consecutive days were well tolerated in chimpanzees.

EXAMPLE XVIII

Clinical Activity and Efficacy of cA2 Antibody

Chimeric IgG1 anti-human TNF MAb cA2 was administered to healthy male human volunteers as patients. One hour after receiving 4 ng/kg of an NIH reference endotoxin, the volunteers were administered either saline, as a control, or 0.01, 0.10 or 10 mg/kg of cA2 in a pharmaceutically acceptable form. TNF levels in serum were measured over time and were found to show a dose dependent decrease in peak TNF levels with no TNF being detected in volunteers receiving a 10 mg/kg dose of cA2. Accordingly, therapy with an anti-TNF antibody of the present invention is expected to inhibit TNF-mediated effects in humans.

Patients receiving endotoxin developed pronounced leukopenia thought to be due to margination of white blood cells. As the white blood cells become activated, they can attach to endothelial receptors with resultant endothelial damage. At doses of 1.0 to 10.0 mg/kg, this leukopenia is prevented, whereas, at 0.01 and 0.1 mg/kg dosages, a drop in white cell count was observed. The drop was most pronounced among the polymorph cell line. In all patients there was a subsequent leukocytosis, which was unchanged by treatment with anti-TNF anti-body cA2. This blocking effect on white blood cell margination is expected to represent a protective effect against the endothelial damage associated with TNF. It is expected in the art that this TNF-related endothelial damage plays a significant role in the morbidity and mortality associated with sepsis, and it is therefore expected that the anti-TNF antibodies of the present invention will provide a protective effect against these damaging effects, as presented herein.

EXAMPLE XIX

Treatment of Sepsis in Humans Using a Chimeric Anti-TNF Antibody

The chimeric anti-TNF MAb cA2 has been used in two phase I/II studies. In a phase I/II study in septic patients, 20 patients with the sepsis syndrome received a single dose of either 0.1, 1.0, 5.0 or 10 milligrams of cA2 per kilogram bodyweight. Another 60 patients received 100 milligrams of HA-1A, a human anti-lipid A Mab currently under evaluation for gram negative sepsis, followed with either placebo or 1.0, 5.0, or 10 milligrams cA2 per kilogram bodyweight. The cA2 was administered as a single, intravenous infusion over a 60 minute period. Clinical assessment, vital signs, and laboratory parameters were measured before, during and periodically for 28 days after the infusion. In this study, cA2 was well tolerated. No adverse events were reported as "probably" or "definitely" related to cA2. All deaths were reported as "definitely not" related to cA2.

Accordingly, human treatment of rheumatoid arthritis in human patients was expected, and found, to provide a suitable treatment, as described herein.

EXAMPLE XX

CLINICAL TREATMENT OF RHEUMATOID ARTHRITIS BY A ANTI-TNF ANTIBODY OR PEPTIDE OF THE PRESENT INVENTION

A Phase I open label study was conducted for methods and compositions of the present invention using a chimeric anti-TNF MAb for the treatment of patients with severe refractory rheumatoid arthritis. Nine patients were enrolled in the study. The first five patients were treated with chimeric anti-TNF antibody (cA2), 10 mg/kg as a single dose infused over a period of two hours. These patients were subsequently retreated with a second infusion of 10 mg/kg on day 14 of the study. The second group of five patients received an infusion of 5 mg/kg on the first day of the study. They were then treated with additional infusions of 5 mg/kg on days 5, 9, and 13. Four of the planned five patients in this second group have been treated to date. Preparation, Administration, and Storage of Test Material The chimeric monoclonal anti-TNF antibody was supplied in single-use glass vials containing 20 mL with 100 mg of anti-TNF (5 mg/mL). The anti-TNF antibody was stored at 2–8° C. Prior to infusion, the antibody was withdrawn from the vials and filtered through a low-protein-binding 0.22 μm filter. This filtered antibody was then diluted to a final volume of 300 mL with normal saline. The 300 mL antibody preparation was then infused via an in-line filter over a period of not less than two hours.

Prior to each repeat infusion of study medication a test dose of 0.1 mL of the infusion was diluted in 10 mL of normal saline and administered by slow IV push over 5 minutes. The patient was observed for 15 minutes for signs or symptoms of an immediate hypersensitivity reaction. If no reaction was observed in this time period, the full dose was administered as described above.

Administration Protocol

Group 1 (patients 1–5): a total of 2 infusions, on day 1 and day 15 of the trial; dosage 10 mg/kg on each occasion;

Group 2 (patients 6–9): a total of 4 infusions, on days 1, 5, 9 and 13 of the trial; dosage 5 mg/kg on each occasion.

All infusions were administered iv over 2 hours in a total volume of cA2+saline of 300 ml. Infusions subsequent to the

first in any patient were preceded by a small test dose administered as an iv push. All patients had at least three years of disease activity with rheumatoid arthritis. The patients ranged in age from 23 to 63. All patients had failed therapy with at least three different DMARD (Disease Modifying Anti-Rheumatic Drug). Six of the nine patients had serum rheumatoid factors, and all nine patients had erosions present on X-rays.

Clinical Monitoring

Patients were monitored during and for 24 hours after infusions for hemodynamic change, fever or other adverse events. Clinical and laboratory monitoring for possible adverse events was undertaken on each follow-up assessment day. Clinical response parameters were performed at the time-points as specified in the flow charts present in Table 9A and Table 9B. These evaluations were performed prior to receiving any infusions.

Clinical response studies will be comprised of the following parameters:

1. Number of tender joints and assessment of pain/tenderness

The following scoring will be used:

0=No pain/tenderness

1=Mild pain. The patient says it is tender upon questioning.

2=Moderate pain. The patient says it is tender and winces.

3=Severe pain. The patient says it is tender and winces and withdraws.

2. Number of swollen joints

Both tenderness and swelling will be evaluated for each joint separately. MCP's, PIP's etc. will not be considered as one joint for the evaluation.

3. Duration of morning stiffness (in minutes)

4. Grip strength

5. Visual analog pain scale (0–10 cm)

6. Patients and blinded evaluators will be asked to assess the clinical response to the drug. Clinical response will be assessed using a subjective scoring system as follows:

5=Excellent response (best possible anticipated response)

4=Good response (less than best possible anticipated response)

3=Fair response (definite improvement but could be better)

2=No response (no effect)

1=Worsening (disease worse)

Measurement of index of disease activity is scored according to the following Table 5.

TABLE 5

Clinical characteristics of patients 1–5

Patient Number	Age/ Sex	Disease Duration (years)	Rheumat. Factor	Erosions/ Nodules	Previous Treatment (DMARDs only)	Concomitant Anti-rheumatic Theraphy
01	48/F	7	+ve	+ve/+ve	*Sal,DP, Myo,Aur, MTX, Aza, Chl.	**Pred 5 mg
02	63/F	7	−ve	+ve/−ve	Sal,Myo, DP.	Para 1–2 g
03	59/M	3	+ve	+ve/−ve	Aur,Chl, Myo,MTX, Sal.	Pred 10 mg Ind 225 mg

TABLE 5-continued

Clinical characteristics of patients 1–5

Patient Number	Age/ Sex	Disease Duration (years)	Rheumat. Factor	Erosions/ Nodules	Previous Treatment (DMARDs only)	Concomitant Anti-rheumatic Theraphy
04	56/M	10	+ve	+ve/−ve	Myo,DP, Aza,Sal.	Pred 12.5 mg Ibu 2 g, Para 1–2 g
05	28/F	3	+ve	+ve/−ve	Myo,Sal, DP,Aza.	Pred 8 mg, Para 1–2 g Cod 16 mg

*Sal = Sulphasalazine; DP = D-penicillamine; Myo = Myocrisin; Aur = auranofin; MTX = methotrexate; Aza = azathioprine; Chl = hydroxychloroquine.

**Pred = prednisolone (dosage/day); Para = paracetamol; Ind = Indomethacin; Ibu-ibuprofen; Cod = codeine phosphate.

TABLE 6

Clinical characteristics of patients 6–9

Patient Number	Age/ Sex	Disease Duration (years)	Rheumat. Factor	Erosions/ Nodules	Previous Treatment (DMARDs only)	Concomitant Anti-rheumatic Theraphy
06	40/M	3	+ve	+ve/−ve	*Sal,Chl, Aur.	**Nap 1 g
07	54/F	7	−ve	+ve/−ve	DP,Myo, Sal,Aza, MTX.	Para 1–2 g Cod 16–32 mg
08	23/F	11	+ve	+ve/−ve	Chl,Myo, Sal,MTX, Aza.	Pred 7.5 mg Dicl 100 mg Para 1–2 g Dext 100–200 mg
09	51/F	15	−ve	+ve/+ve	Myo,Chl, DP,MTX.	Pred 7.5 mg Dicl 125 mg, Para 1–3 g

*Sal = Sulphasalazine; Chl = chloroquine or hydroxychloroquine; Aur = auranofin; DP = D-penicillamine; Myo = Myocrisin; Aza = azathioprine; MTX = methotrexate.

**Nap = naprosyn (dosage/day); Para = paracetamol; Cod = codeine phosphate; Pred = prednisolone; Dicl = diclofenac; Dext = dextropropoxyphene.

TABLE 7

Disease activity at entry for patients 1–5

Patient IDA Number	Morning Stiffness (mins)	Pain (0–10 cm on VAS)	Number Swollen Joints (0–28)	Ritchie Articular Index (0–69)	Grip Strength L/R (mm/Hg; max 300)	ESR (mm/hr normal ranges: F < 15; M < 10)	CRP (mg/l; normal range: < 10)	(range: 1–4)
01	60	3.9	19	30	108/107	35	5	2.67
02	20	2.7	25	31	67/66	18	14	2.0
03	90	4.9	14	16	230/238	48	44	2.5
04	30	6.9	17	12	204/223	24	35	2.33
05	90	5.7	28	41	52/89	87	107	3.0

TABLE 8

Disease activity at entry for patients 6–9

Patient IDA Number	Morning Stiffness (mins)	Pain (0–10 cm on VAS)	Number Swollen Joints (0–28)	Ritchie Articular Index (0–69)	Grip Strength L/R (mm/Hg; max 300)	ESR (mm/hr normal ranges: F < 15; M < 10)	CRP (mg/l; normal range: < 10)	(range: 1–4)
06	120	5.0	3	4	260/280	23	33	2.33
07	105	7.4	27	31	59/80	25	10	2.83
08	270	9.3	17	37	73/125	35	31	3.17
09	180	4.5	20	26	53/75	15	33	2.5

All patients have tolerated the infusions of chimeric anti-CD4 and no serious adverse reactions have been observed. Specifically, no episodes of hemodynamic instability, fevers, or allergic reactions were observed in association with the infusions. Patients have not experienced any infections.

Although this is a non-blinded study, all patients experienced improvement in their clinical assessments of disease status, as well in biochemical parameters of inflammation measured in their serum.

Clinical assessments, including the duration of early morning stiffness; the assessment of pain on a visual analogue scale; total count of swollen joints; Ritchie articular index (a scaled score which assesses the total number of tender joints and the degree of joint tenderness); and Index of Disease Activity (a scaled score which incorporates several clinical and laboratory parameters), showed impressive improvements compared to controls. These improvements were typically in the range of an 80% drop from the baseline score; a degree of improvement which is well beyond the amount of improvement that can be attributed to placebo response. In addition, the duration of these improvements was for six to eight weeks in most cases, a duration of response far longer than would be anticipated from a placebo.

The improvements in clinical assessments were corroborated by improvements in biochemical inflammatory parameters measured in serum. The patients showed rapid drops of serum C-reactive protein, usually in the range of 80% from the baseline. Reductions in the erythrocyte sedimentation rate, usually in the range of 40%, were also observed. Circulating soluble TNF receptors were also decreased following therapy. The durations of the biochemical responses were similar to the duration of the clinical responses.

Preliminary assessment of immune responses to the chimeric anti-TNF antibody has shown no antibody response in the first four patients.

In summary, the preliminary evaluation of the results of this Phase I trial indicate that treatment of patients with advanced rheumatoid arthritis with anti-TNF MAb of the present invention is well tolerated and anti-TNF treatment is associated with rapid and marked improvement in clinical parameters of disease activity, including early morning stiffness, pain, and a number of tender and swollen joints; and is accompanied by improvement of biochemical parameters of inflammation.

Although this was an open label study, the magnitude of the clinical improvements is well beyond the degree of improvement that would be anticipated from a placebo response, such that the present invention is shown to have significant clinical efficacy for treating rheumatoid arthritis.

TABLE 9A

Flowchart for CHIMERIC ANTI-TNF STUDY C0168TRA
Group I (10 mg/kg at day 1 and day 14)

	Pre Scr	Screening	Wk d1	0 d2	Wk 1	Wk 2 d14	Wk 3	Wk 4	Wk 6	Wk 8
Consent	x									
Demography		x								
Physical Examination		x								x
Pregnancy Test		x								
Weight		x	x			x				x
Vital Signs		x	x*	x	x	x*	x	x	x	x
Anti-TNF Infusion			x			x				
Labs, see Chart		x	x'	x	x	x'	x	x	x	x
Clinical (Safety)				x	x	x'	x	x	x	x
Clinical (Response)		x	x'		x	x'	x	x	x	x
Synovial biopsy		x			x7					
Response evaluation										x

	Screening	Wk d1	0 d2	Wk 1	Wk 2 d14	Wk 3	Wk 4	Wk 6	Wk 8	
Hematology + ESR	x	x'		x	x'	x	x	x	x	
Biochemistry	x	x'		x	x'	x	x	x	x	
Urinalysis		x'		x	x'	x	x	x	x	
CRP + RF		x'		x	x'	x	x	x	x	
Serum Cytokines		x'		x	x'	x	x	x	x	
PBL		x	x	x						
Pharmacokinetics		x#	x#		x$					
HACA response		x'		x	x'	x	x	x	x	x

x* = vital signs will be obtained prior to infusion, every 30 minutes during the infusion and every 30 minutes for 2 hours after the infusion.
x' = Needs to be done prior to the infusion.
x# = Serum samples will be obtained prior to the infusion and at 1, 2, 4, 8, 12, and 24 hours after the end of the infusion.
x$ = Serum samples will be obtained to the infusion and at 2 hours

TABLE 9B

Flowchart for CHIMERIC ANTI-TNF STUDY C0168TRA
Group II2 (mg/kg every 4 days, 4 times total)

	Pre Scr	Screening	d1	wk d2	0 d5	+ d9	1 d13	wk 2	wk 3	wk 4	wk 6	wk 8	
Consent	x												
Demography		x											
Physical exam		x										x	
Pregnancy test		x											
Weight		x	x		x	x	x					x	
Vital signs		x	x+	x	x*	x*	x*	x	x	x	x	x	
Anti-TNF Infusion			x		x	x	x						
Labs, see chart		x	x'	x	x'	x'	x'	x	x	x	x	x	x
Clinical Safety				x	x'	x'	x'	x	x	x	x	x	
Clinical Response		x	x'			x'		x	x	x	x	x	
Synovial Biopsy		x					x7						
Response Evaluation												x	
Hematology + ESR		x	x'			x'		x	x	x	x	x	
Biochemistry		x	x'			x'		x	x	x	x	x	
Urinalysis			x'			x'		x	x	x	x	x	
CRP + RF			x'			x'		x	x	x	x	x	
Cytokines			x'			x'		x	x	x	x	x	
PBL			x	x		x				x			

TABLE 9B-continued

Flowchart for CHIMERIC ANTI-TNF STUDY C0168TRA
Group II2 (mg/kg every 4 days, 4 times total)

	Pre Scr	Screening	d1	wk d2	0 d5	+ d9	1 d13	wk 2	wk 3	wk 4	wk 6	wk 8
Pharmaco-kinetics			x#	x#	x$	x$	x$					
HACA Response			x'			x'		x	x	x	x	x

x* = Vital signs will be obtained prior to infusion, every 30 minutes during the infusion and every 30 minutes for 2 hours after infusion.
x' = Needs to be done prior to the infusion.
x# = Serum samples will be obtained to the infusion and at 1, 2, 4, 8, 12, and 24 hours after the end of the infusion.
x$ = Serum samples will be obtained prior to the infusion and at 2 hours after the end of the infusion.

TABLE 10

Measurement of the index of disease activity (DA)
Variables of Disease Activity

IDA score	Morning Stiffness (min)	Pain (VAS, cm)*	Grip Strength (mm-Hg)	Ritchie Articular Index	Hemoglobin (g/dl) Male	Hemoglobin (g/dl) Female	ESR
1	<10	0–2.4	>200	0	>14.1	>11.7	0–20
2	10–30	2.5–4.4	50–200	1–7	13–14	10.8–11.6	21–45
3	31–120	4.5–6.4	30–49	8–17	10–12.9	8.4–10.7	46–80
4	>120	6.5–10	<30	>18	<9.9	<8.3	>81

*Pain was measured on a visual analog scale (VAS) 0–10 cm.

Conclusions (1)

Safety of anti-TNF in RA

Anti-TNF was safe and very well tolerated:

no hemodynamic, febrile or allergic episodes;

no infections;

no clinical adverse events;

a single laboratory adverse event only, probably unrelated to anti-TNF.

Conclusions (2)

Efficacy of anti-TNF in RA

Anti-TNF therapy resulted in:

rapid and marked improvements in EMS, pain and articular index in most patients;

slower but marked improvement in swollen joint score, maximal by 3–4 weeks;

rapid and impressive falls in serum CRP, and a slower fall in ESR;

normalization of CRP and ESR in some patients;

rapid falls in serum C4d (a complement breakdown product) and IL-6 in patients where these indices were elevated at entry.

Duration of clinical improvements variable, with rebound in some patients at 6–8 weeks.

Accordingly, the present invention has been shown to have clinical efficacy in human patients for treating TNF involved pathologies using TNF MAbs of the present invention, such as for treating rheumatoid arthritis. Additionally, the human clinical use of TNF antibodies of the present invention in humans is also shown to correlate with in vitro data and in vivo animal data for the use of anti-TNF antibodies of the present invention for treating TNF-related pathologies.

EXAMPLE XXI

TREATMENT OF CROENIS DISEASE IN HUMANS USING ANTI-TNFα ANTIBODIES

Case History SB.

This 16 year old patient has a history of Crohn's disease since age 12. She was suffering from diarrhoea, rectal blood loss, abdominal pain, fever and weight loss. She showed perianal lesions, severe colitis and irregularity of the terminal ileum. She was treated with prednisolone (systemic and local) and pentasa. This resulted in remission of the disease, but she experienced extensive side effects of the treatment. She experienced severe exacerbations at age 12 and 12 yrs, 5 months, (Immuran™ added), 12 yrs, 9 months, 13 yrs, 5 months, and 14 yrs, 10 months. She experienced severe side effects (growth retardation, morbus Cushing, anemia, muscle weakness, delayed puberty, not able to visit school).

At 15 yrs, 11 months, she was diagnosed with a mass in the right lower quadrant. She had a stool frequency of 28 time per week (with as much as 10 times per day unproductive attempts). The Crohn's index wad 311, the pediatric score 77.5. The sedimentation rate was elevated. Albumen and hemoglobin reduced. Before the first treatment the score was 291 and pediatric score was 60, and she would possibly have to loose her colon. She was infused on compassionate grounds with 10 mg/kg cA2, without any side effects noticed. One week after treatment her sedimentation rate was reduced from 66 to 32 mm. The Crohn's index was 163 and pediatric score 30. She was reported to feel much better and the frequency of the stools was reduced greatly. Thee was apparently no more diarrhoea, but normal faeces. On October 15th, before the second infusion she had gained weight, had a sedimentation rate of 20 mm, an albumen of 46 h/l, Crohn's index 105, pediatric score 15. There seemed to be improvement on video endoscopy. A second infusion was performed at 16 yrs.

The patient was greatly improved after the second infusion. A endoscopy showed only 3 active ulcers and scar tissue.

This is in contrast with her colon on admission when the thought was that her colon should be removed. This case history shows a dramatic improvement of severe Crohn's disease upon treatment with cA2 anti-TNF antibody.

TABLE 11

CASE HISTORY SB

11 y, 8 m:	physical examination:	Diarrhoea, rectal blood loss, abdominal pain, fever (40°) weight loss perianal lesions
	sigmoidoscopy:	severe colitis, probably M. Crohn
	enterolysis:	irregularity terminal ileum
	Therapy:	prednisolone 10 mg 3 dd. Pentasa 250 mg 3 dd. enema (40 mg prednisone, 2 g 5 ASA) ml 1 dd.
	Result:	remission, however: extensive side effects of prednisone and stunting growth
	Action:	prednisone
11 y, 11 m		exacerbation same clinical picture as 11 y, 8 m
	sigmoidoscopy:	recurrence of colitis (grade IV) in last 60 cm and anus.
	Therapy:	prednisolone 40 mg 1 dd Pentasa 500 mg 3 dd enema 1 dd
	Result:	better
12 y, 5 m:		severe exacerbation; despite intensive treatment
	sigmoidoscopy:	extensive perianal and sigmoidal lesions; active disease
	Therapy:	continued + Immuran ™ 25 mg 1 dd
	Result:	slight improvement however still growth retardation, cushing, anaemia, muscle weakness.
	Action:	prednisone
12 y, 9 m:		exacerbation
	sigmoidoscopy:	extensive (active) colitis, polyps
	Action:	prednisone: 30 mg 1 dd, Immuran ™ 50 mg 1 dd, Pentasa 500 mg 3 dd, enema 2 dd
	Result:	still needs enema's with prednisone and oral prednisone. delayed puberty, stunting growth
14 y, 10 m:		severe exacerbation, weight loss, abdominal pain, fever.
	ileoscopy:	active colitis (grade IV), perianal lesions. Terminal ileum normal.
	Result:	No remission still fever, poor appetite, weight loss, diarrhea, not able to visit school
Important Findings:		
14 y, 11 m:		151.9 cm: 34 kg T = 38° C., Abdominal mass in right lower quadrant stool frequency 28 per week (however goes 10–15 times a day but most often without success) ESR 55 mm; Hb 6.2 mmol/l Ht 0, 29 1/1; alb. 38.4 g/l Crohn's Dis. Act. Index: 311 Pediatric score: 77.5
14 y, 11.2 m: (before 1st infusion)	Crohn's Dis Act Index:	151, 8 cm: 34.6 kg 291
	Pediatric score:	60
14 y, 11.4 m:		151, 8 cm: 34.6.kg ESR 32 mm: Hb 5.7 mmol/l
	Crohn's Dis Act Index:	163
	pediatric score:	30
15 y, 0 m (before 2nd infusion		152,1 cm: 34.8 kg Feels like she has never felt before. Parents also very enthusiastic ESR 30 mm: Hb 6, 3 mol/l Ht 0, 32 11; Alb 46 g/l
	Crohn Dis Act Index:	105
	Pediatric Score:	15
	Video-endoscopy:	Improvement

No problems or side effects observed during and following infusion.

Accordingly, anti-TNF antibodies according to the present invention, as exemplified by cA2, are shown to provide successful treatment of TNF related pathologies, as exemplified by Crohn's disease, in human patients with no or little side effects.

EXAMPLE XXII

TREATMENT OF ARTHRITIS IN HUMANS USING CHIMERIC IMMUNOGLOBULIN CHAIN OF THE PRESENT INVENTION

Patient Selection

Twenty patients were recruited, each of whom fulfilled the revised American Rheumatism Association criteria for the diagnosis of RA (Arnett et al., *Arthritis Rheum*. 31:315–324 (1988). The clinical characteristics of the patients are shown in Table 12. The study group comprised 15 females and 5 males, with a median age of 51 years (range 23–72), a median disease duration of 10.5 years (range 3–20) and a history of failed therapy with standard disease-modifying anti-rheumatic drugs (DMARDs; median number of failed DMARDs: 4, range 2–7). Seventeen were seropositive at entry or had been seropositive at some stage of their disease, all had erosions on X-Rays of hands or feet, and 3 had rheumatoid nodules. All patients had active disease at trial entry, as defined by an Index of Disease Activity (IDA; Mallya et al., *Rheumatol. Rehab*. 20:14–17 (1981) of at least 1.75, together with at least 3 swollen joints, and were classed as anatomical and functional activity stage 2 or 3 (Steinbrocker et al., *JAMA* 140:659–662 (1949). The pooled data for each of the clinical and laboratory indices of disease activity at the time of screening for the trial (up to 4 weeks prior to trial entry), and on the day of trial entry itself (week 0), are shown in Tables 13 and 14.

TABLE 12

Demographic features of 20 patients with refractory rheumatoid arthritis.

Patient	Age/ Sex	Disease Duration (years)	Previous DMARDs	Concomitant Therapy
1	48/F	7	SSZ, DP, GST, AU RMTX, AZA, HCQ	Pred 5 mg
2	63/F	7	SSZ, GST, DP	Para 1–2 g
3	59/M	3	AUR, HCQ, GST, MTX, SSZ	Pred 10 mg, Indo 225 mg
4	56/M	10	GST, DP, AZA, SSZ	Pred 12.5 mg, Ibu 2 g, Para 1–2 g
5	28/F	3	GST, SSZ, DP, AZA	Pred 8 mg, Para 1–2 g, Cod 16 mg

TABLE 12-continued

Demographic features of 20 patients with refractory rheumatoid arthritis.

Patient	Age/ Sex	Disease Duration (years)	Previous DMARDs	Concomitant Therapy
6	40/M	3	SSZ, HCQ, AUR	Nap 1 g
7	54/F	7	DP, GST, SSZ, AZA MTX	Para 1–2 g, Cod 16–32 mg
8	23/F	11	HCQ, GST, SSZ, MTX AZA	Pred 7.5 mg, Dicl 100 mg, Para 1–2 g, Dex 100–200 mg
9	51/F	15	GST, HCQ, DP, MTX	Pred 7.5 mg, Dicl 125 mg, Para 1–3 g
10	47/F	12	SSZ, CYC, MTX	Ben 4 g
11	34/F	10	DP, SSZ, MTX	Pred 10 mg, Para 1.5 g, Cod 30–90 mg
12	57/F	12	GST, MTX, DP, AUR	Asp 1.2 g
13	51/F	7	SSZ, AZA	Para 1–4 g
14	72/M	11	GST, DP, AZA, MTX	Pred 5 mg, Para 1–4 g, Cod 16–64 mg
15	51/F	17	HCQ, DP, SSZ, MTX	Asp 0.3 g
16	62/F	16	GST, DP, SSZ, MT X AZA	Para 1–4 g, Cod 16–64 mg
17	56/F	11	SSZ, DP, GST, MTX HCQ, AZA	Pred 7.5 mg, Eto 600 mg, para 1–2 g, Dext 100–200 mg
18	48/F	14	GST, MTX, DP, SSZAUR, AZA	Pred 7.5 mg, Indo 100 mg, Para 1–3 g
19	42/F	3	SSZ, MTX	Fen 450 mg, Ben 6 g, Cod 30 mg
20	47/M	20	GST, DP, SSZ, AZA	Pred 10 mg, Para 1–3 g

*DMARDs = disease - modifying anti-rheumatic drugs SSZ = sulphasalazine; DP = D-penicillamine; GST = gold sodium thiomalate; AUR = auranofin; MTX = methotrexate; AZA = azathioprine; HCQ = (hydroxy) chloroquine; CYC = cyclophosphamide. Pred = prednisolone (dose/day); Para = paracetamol; Indo = Indomethacin; Ibu = ibuprofen; Cod = codeine phosphate; Nap = naprosyn; Dicl = diclofenac; Dext = dextropropoxyphene; Ben = benorylate; Asp = asprin; eto = etodolac; Fen = fenbufen.

TABLE 13

Changes in clinical assessments following treatment of rheumatoid arthritis patients with cA2.

Week of Trial	Morning Stiffness min	Pain Score (0–10) cm	Ritchie Index (0–69)	Swollen Joints (0–28) number	Grip Strength (L) (0–300) mm Hg	Grip Strength (R) (0–300) mm Hg	IDA (1–4)	Patient Assessment (grades improved 0–3)
Screen	135(0–600)	7.4(4–9.7)	23(4–51)	16(4–28)	84(45–300)	96(57–300)	3(2.3–3.3)	NA
p value 0	180(20–600)	7.1(2.7–9.7)	28(4–52)	18(3–27)	77(52–295)	92(50–293)	3(2–3.5)	NA
p value 1	20(0–180) <0.001	2.6(0.6–7.8) <0.001	13(2–28) <0.001; <0.002	13.5(1–25) >0.05	122(66–300) >0.05	133(57–300) >0.05	2(1.5–3.3) <0.001	1(1–3) NA
p value 2	15(0–150) <0.001	3.0(0.3–6.4) <0.001	13(1–28) <0.001	11.5(1–22) <0.003; <0.02	139(75–300) <0.03; >0.05	143(59–300) >0.05	2(1.5–3.2) <0.001	1.5(1–3) NA
p value 3	5(0–150) <0.001	2.2(0.2–7.4) <0.001	8(0–22) <0.001	6(1–19) <0.001; <0.002	113(51–300) >0.05	142(65–300) >0.05	2(1.2–3.2) <0.001	2(1–2) NA
p value 4	15(0–90) <0.001	1.9(0.1–5.6) <0.001	10(0–17) <0.001	6(0–21) <0.001; <0.002	124(79–300) <0.02; >0.05	148(64–300) <0.03; >0.05	1.8(1.3–2.7) <0.001	2(1–2) NA
p value 6	5(0–90) <0.001	1.9(0.1–6.2) <0.001	6(0–18) <0.001	5(1–14) <0.001	119(68–300) <0.04; >0.05	153(62–300) <0.05; >0.05	1.7(1.3–2.8) <0.001	2(1–2) NA
p value 8	15(0–60) <0.001	2.1(0.2–7.7) <0.001	8(1–28) <0.001	7(1–18) <0.001	117(69–300) <0.03; >0.05	167(53–300) <0.03; >0.05	1.8(1.5–2.8) <0.001	2(1–3) NA

Datas are expressed as the median (range) of values from 20 patients; data from patient 15 were not included after week 2 (dropout); P values show significance by Mann-Whitney test compared with week 0 values; adjusted for multiple statistical comparisons. IDA = Index of disease activity; NA = not applicable.

TABLE 14

Changes in laboratory measures following treatment of rheumatoid arthritis patients with cA2.

Week of Trial	Hgb g/liter	WBC × 10/liter	Platelet Count × 10/liter	ESR mm/hour	CRP mg/liter	SAA mg/ml	RF Inverse titer
Screen	117(98–146)	7.9(3.9–15.2)	352(274–631)	59(18–87)	42(9–107)	ND	ND
p value 0	113(97–144)	9.0(4.9–15.7)	341(228–710)	55(15–94)	39.5(5–107)	245(18–1900)	2,560 (160–10,240)
p value 1	114(96–145) >0.05	8.5(3.6–13.6) >0.05	351(223–589) >0.05	26(13–100) >0.05	5(0–50) <0.001	58(0–330) <0.001; <0.003	ND
p value 2	112(95–144) >0.05	8.2(4.3–12.7) >0.05	296(158–535) <0.04; >0.05	27(10–90) <0.02; >0.05	5.5(0–80) <0.001; <0.003	80(11–900 <0.02; <0.04	ND
p value 3	110(89–151) >0.05	9.0(3.7–14.4) >0;05	289(190–546) <0.03; >0,05	27(12–86) <0.04; >0.05	7(0–78) <0.001; <0.002	ND	ND
p value 4	112(91–148) >0.05	8.2(4.7–13.9) >0.05	314(186–565) >0.05	23(10–87) <0.04; >0.05	10(0–91) <0.004; <0.02	ND	ND
p value 6	116(91–159 >0.05	9.1(2.9–13.9) >0.05	339(207–589) >0.05	23(12–78) <0.03; >0.05	8(0–59) <0.001	ND	ND
p value 8	114(94–153) >0.05	7.6(4.2–13.5) >0.05	339(210–591) >0.05	30(7–73) >0.05	6(0–65) <0.001	ND	480(40–05,120) >0.05

Data are expressed as the median (range) of values from 20 patients; data from patient 15 were not included after week 2 (dropout). For rheumatoid factor (RF), only those patients with week 0 titers > 1/160 in the particle agglutination assay were included (No. = 14). P values show significance by Mann-Whitney test compared with week 0 values; adjusted for multiple statistical comparisons; ND = not done. Normal ranges: hemoglobin (Hgb) 120–160 g/liter (F), 135–175 g/liter (M); white blood cell count (WBC) 4–11 × 10^9/liter; platelet count 150–400 × 10^9/liter; erythrocyte sedimentation rate (ESR) < 15 mm/hour (F), < 10 mm/hour (M); C-reactive protein (CRP) < 10 mg/liter; serum amyloid A (SAA) < 10 mg/ml.

TABLE 16

260793	270793	280793	290793	020893	200893	270893
ESR-77	ESR-47	BSR-58	ESR-77	ESR-77	ESR-46	ESR-38

All DMARDs were discontinued at least 1 month prior to trial entry. Patients were allowed to continue on a non-steroidal anti-inflammatory drug and/or prednisolone (<12.5 mg/day) during the trial. The dosage of these agents was kept stable for 1 month prior to trial entry and during the course of the trial, and no parenteral corticosteroids were allowed during these periods. Simple analgesics were allowed ad libitum. Patients with other serious medical conditions were excluded. Specific exclusions included serum creatinine>150 umol/liter (normal range 60–120 umol/liter), hemoglobin (Hgb)<90 gm/liter (normal range 120–160 gm/liter [females]; 135–175 gm/liter [males]), white blood cell count (WBC)<4×10^9/liter (normal range 4–11×10^9/liter), platelet count<100×10^9/liter (normal range 150–400×10^9/liter), and abnormal liver function tests or active pathology on chest X-Ray.

All patients gave their informed consent for the trial, and approval was granted by the local ethics committee.

Treatment

The cA2 antibody was stored at 4° C. in 20 ml vials containing 5 mg of cA2 per milliliter of 0.01 M phosphate buffered saline in 0.15M sodium chloride at a pH of 7.2 and was filtered through a 0.2 um sterile filter before use. The appropriate amount of cA2 was then diluted to a total volume of 300 ml in sterile saline and administered intravenously via a 0.2 um in-line filter over a 2 hour period.

Patients were admitted to hospital for 9–24 hours for each treatment, and were mobile except during infusions. The trial was of an open, uncontrolled design, with a comparison of two treatment schedules. Patients 1 to 5 and 11 to 20 received a total of 2 infusions, each of 10 mg/kg cA2, at entry to the study (week 0) and 14 days later (week 2). Patients 6 to 10 received 4 infusions of 5 mg/kg activity included complete blood counts, C-reactive protein (CRP; by rate nephelometry) and the erythrocyte sedimentation rate (ESR; Westergren). Follow-up assessments were made at monthly intervals after the conclusion of the formal trial period, in order to assess the duration of response.

Analysis of improvement in individual patients was made using two separate indices. Firstly, an index of disease activity (IDA) was calculated for each time point according to the method of Mallya and Mace (Mallya et al., *Rheumatol. Rehab.* 20:14–17 (1981), with input variable of morning stiffness, pain score, Ritchie Index, grip strength, ESR and Hgb. The second index calculated was that of Paulus (Paulus et al., *Arthritis Rheum.* 33:477–484 (1990) which uses input variables of morning stiffness, ESR, joint pain/tenderness, joint swelling, patient's and physician's global assessment of disease severity. In order to calculate the presence or otherwise of a response according to this index, two approximations were made to accommodate our data. The 28 swollen joint count used by us (nongraded; validated in Fuchs et al., *Arthritis Rheum.* 32:531–537 (1989)) was used in place of the more extensive graded count used by Paulus, and the patient's and physician's global assessments of response recorded by us were approximated to the global assessments of disease activity used by Paulus infra. In addition to determining response according to these published indices, we selected 6 disease activity assessments of interest (morning stiffness, pain score, Ritchie index, swollen joint count, ESR and CRP) and calculated their mean percentage improvement. We have used FIGS. **24** and **25** to give an indication of the degree of improvement seen in responding patients.

Immunological Investigations—Rheumatoid factors were measured using the rheumatoid arthritis particle agglutination assay (RAPA, FujiBerio Inc., Tokyo, Japan), in which titers of 1/160 or greater were considered significant. Rheumatoid factor isotypes were measured by ELISA (Cambridge Life Sciences, Ely, UK). The addition of cA2 at concentrations of up to 200 ug/ml to these assay cA2, at entry, and days 4, 8 and 12. The total dose received by the 2 patient groups was therefore the same at 20 mg/kg.

Assessment

Safety Monitoring—Vital signs were recorded every 15 to 30 minutes during infusions, and at intervals for up to 24 hours post infusion. Patients were questioned concerning possible adverse events before each infusion and at weeks 1, 2, 3, 4, 6, and 8 of the trial. A complete physical examination was performed at screening and week 8. In addition, patients were monitored by standard laboratory tests including complete blood count, C3 and C4 components of complement, IgG, IgM and IgA, serum electrolytes, creatinine, urea, alkaline phosphatase, aspartate transaminase and total bilirubin. Sample times for these tests were between 0800 and 0900 hours (pre-infusion) and 1200–1400 hours (24 hours post completion of the infusion). Blood tests subsequent to 15 day 1 were performed in the morning, usually between 0700 and 1200 hours. Urine analysis and culture were also performed at each assessment point.

Response Assessment—The patients were assessed for response to cA2 at weeks 1, 2, 3, 4, 6 and 8 of the trial. the assessments were all made between 0700 and 1300 hours by the same observer. The following clinical assessments were made: duration of morning stiffness (minutes), paid score (0 to 10 cm on a visual analog scale), Ritchie Articular Index (maximum 69; Ritchie et al., *Quart. J. Med.* 147:393–406 (1968)), number of swollen joints (28 joint count; validated in Fuchs et al., *Arthritis Rheum.* 32:531–537 (1989), grip strength (0 to 300 mm Hg, mean of 3 measurements per hand by sphygmomanometer cuff) and an assessment of function (the Stanford Health Assessment Questionnaire (HAG) modified for British patients; 34). In addition, the patients' global assessments of response were recorded on a 5-point scale (worse, no response, fair response, good response, excellent response). Routine laboratory indicators of disease systems did not alter assay results (data not shown). Antinuclear antibodies were detected by immunofluorescence on HEpo 2 cells (Biodiagnostics, Upton, Worcs. UK) and antibodies to extractable nuclear antigens were measured by counter immunoelectrophoresis with poly-antigen extract (Biodiagnostics). Sera positive by immunofluorescence were also screened for antibodies to DNA by the Farr assay (Kodak Diagnostics, Amersham, UK). Anti-cardiolipin antibodies were measured by ELISA (Shield Diagnostics, Dundee, Scotland). Serum amyloid A (SAA) was measured by sandwich ELISA (Biosource International, Camarillo, Calif., USA). Antiglobulin responses to the infused chimeric antibody were measured by an in-house ELISA, using cA2 as a capture reagent.

Cytokine Assays—Bioactive TNF was measured in sera using the WEHI 164 clone 13 cytotoxicity assay (Espevik et al., *J. Imm. Methods* 95:99–105 (1986). Total IL-6 was measured in sera using a commercial immunoassay (Medgenix Diagnostics, SA, Belgium) and by a sandwich ELISA developed 'in house' using monoclonal antibodies provided by Dr. F. di Padova (Basel, Switzerland). Microtiter plates were coated with monoclonal antibody LNI 314-14 at a concentration of 3 ug/ml for 18 hours at 4° C. and blocked with 3% bovine serum albumin in 0.1M phosphate buffered saline, pH 7.2. Undiluted sera or standards (recombinant hIL-6, 0–8.1 ug/ml) were added to the wells in duplicate and incubated for 18 hours at 4° C. Bound IL-6 was detected by incubation with monoclonal antibody LNI 110-14 for 90 minutes at 37° C., followed by biotin—labeled goat anti-murine IgG2b for 90 minutes at 37° C. (Southern Biotechnology, Birmingham, Ala.). The assay was developed using streptavidin—alkaline phosphatase (Southern Biotechnology) and p-nitrophenylphosphate as a substrate and the optical density read at 405 nm.

Statistics—Comparisons between week 0 and subsequent time points were made for each assessment using the Mann-Whitney test. For comparison of rheumatoid factor (RAPA) titers, the data were expressed as dilutions before applying the test.

This was an exploratory study, in which pre-judgements about the optimal times for assessment were not possible. Although it has not been common practice to adjust for multiple statistical comparisons in such studies, a conservative statistical approach would require adjustment of p values to take into account analysis at several time points. The p values have therefore been presented in two forms: unadjusted, and after making allowance for analysis at multiple time points by use of the Bonferroni adjustment. Where p values remained <0.001 after adjustment, a single value only is given. A p value of <0.05 is considered significant.

Results

Safety of cA2—the administration of cA2 was exceptionally well tolerated, with no headache, fever, hemodynamic disturbance, allergy or other acute manifestation. No serious adverse events were recorded during the 8-week trial. Two minor infective episodes were recorded, patient 15 presented at week 2 with clinical features of bronchitis and growth of normal commensals only on sputum culture. She had a history of smoking and of a similar illness 3 years previously. The illness responded promptly to treatment with amoxicillin, but her second cA2 infusion was withheld and the data for this patient are therefore not analyzed beyond week 2. Patient 18 showed significant bacteriuria on routine culture at week 6($>10^5$/ml; lactose fermenting coliform), but was asymptomatic. This condition also responded promptly to amoxicillin.

Routine analysis of blood samples showed no consistent adverse changes in hematological parameters, renal function, liver function, levels of C3 or C4 or immunoglobulins during the 8 weeks of the trial. Four minor, isolated and potentially adverse laboratory disturbances were recorded. Patient 2 experienced a transient rise in blood urea, from 5.7 mmol/liter to 9.2 mmol/liter (normal range 2.5 to 7 mmol/liter), with no change in serum creatinine. This change was associated with the temporary use of a diuretic, prescribed for a non-rheumatological disorder. The abnormality normalized within 1 week and was classified as 'probably not' related to cA2. Patient 6 experienced a transient fall in the peripheral blood lymphocyte count, from 1.6 to 0.8×10^9/liter (normal range 1.0–4.8×10^9/liter). This abnormality normalized by the next sample point (2 weeks later), was not associated with any clinical manifestations and was classified as 'possible related' to cA2. Patients 10 and 18 developed elevated titers of anti-DNA antibodies at weeks 6 and 8 of the trial, with elevated anti-cardiolipin antibodies being detected in patient 10 only. Both patients had a pre-existing positive antinuclear antibody and patient 10 had a history of borderline lymphocytopenia and high serum IgM. There were no clinical features of systemic lupus erythematosus and the laboratory changes were judged 'possibly related' to cA2.

Efficacy of cA2

Disease Activity—The pattern of response for each of the clinical assessments of disease activity and the derived IDA are shown in Table 13. All clinical assessments showed improvement following treatment with cA2, with maximal responses from week 3. Morning stiffness fell from a median of 180 minutes at entry to 5 minutes at week 6 ($p<0.001$, adjusted), representing an improvement of 73%. Similarly, the Ritchie Index improved from 28 to 6 at week 6, ($p<0.001$, adjusted, 79% improvement) and the swollen joint count fell from 18 to 5, ($p<0.001$, adjusted, 72% improvement). The individual swollen joint counts for all time points are shown in FIG. **24**. Grip strength also improved; the median grip strength rose from 77 (left) and 92 (right) mm Hg at entry to 119 (left) and 153 (right) mmHg at week 6 ($p<0.04$, $p<0.05$, left and right respectively; $p>0.05$ after adjustment for multiple comparisons). The IDA showed a fall from a median of 3 at entry to 1.7 at week 6 ($p<0.001$, adjusted). Patients were asked to grade their responses to cA2 on a 5 point scale. No patient recorded a response of 'worse' or 'no change' at any point in the trial. 'fair', 'good' and 'excellent' responses were classed as improvements of 1, 2 and 3 grades respectively. At week 6, the study group showed a median of 2 grades of improvement (Table 13).

We also measured changes in the patients' functional capacity, using the HAQ modified for British patients (range 0–3). The median (range) HAQ score improved from 2(0.9–3) at entry to 1.1 (0–2.6) by week 6, ($p<0.001$; $p<0.002$ adjusted).

The changes in the laboratory tests which reflect disease activity are shown in Table 14. the most rapid and impressive changes were seen in serum CRP, which fell from a median of 39.5 mg/liter at entry to 8 mg/liter by week 6 of the trial ($p<0.001$, adjusted; normal range<10 mg/liter), representing an improvement of 80%. Of the 19 patients with elevated CRP at entry, 17 showed falls to the normal range at some point during the trial. The improvement in CRP was maintained in most patients for the assessment period (Table 14 and FIG. **25**); the exceptions with high values at 4 and 6 weeks tended to be those with the highest starting values (data not shown). The ESR also showed improvement, with a fall from 55 mm/hour at entry to 23 mm/hour at week 6 ($p<0.03$; $p>0.05$ adjusted; 58% improvement; normal range<10 mm/hour, <15 mm/hour, males and females respectively). SAA levels were elevated in all patients at trial entry, and fell from a median of 245 mg/ml to 58 mg/ml at week 1 ($p<0.003$, adjusted; 76% improvement; normal range<10/mg/ml) and to 80 mg/ml at week 2 ($p<0.04$, adjusted). No significant changes were seen in Hgb, WBC or platelet count at week 6, although the latter did improve at weeks 2 and 3 compared with trial entry (Table 14).

The response data have also been analyzed for each individual patient. The majority of patients had their best overall responses at week 6, at which time 13 assessed their responses as 'good' while 6 assessed their responses as 'fair'. Eighteen of the 19 patients who completed the treatment schedule achieved an improvement in the index of Disease Activity (Mallya et al., *Rheumatol. Rehab.* 20:14–17 (1981) of 0.5 or greater at week 6, and 10 achieved an improvement of 1.0 or greater. All patients achieved a response at week 6 according to the index of Paulus (Paulus et al., *Arthritis Rheum.* 33:477–484 (1990). Finally, all patients showed a mean improvement at week 6 in the 6 selected measures of disease activity (as presented above) of 30% or greater, with 18 of the 19 patients showing a mean improvement of 50% or greater.

Although the study was primarily designed to assess the short-term effects of cA2 treatment, follow-up clinical and laboratory data are available for those patients followed for sufficient time (number=12). The duration of response in these patients, defined as the duration of a 30% (or greater) mean improvement in the 6 selected disease activity measures, was variable, ranging from 8 to 25 (median 14) weeks.

Comparison of the clinical and laboratory data for patients treated with 2 infusions of cA2 (each at 10 m/kg) compared with those treated with 4 infusions (each at 5 mg/kg) showed no significant differences in the rapidity or extent of response (data not shown).

Inununological Investigations and cytokines—Measurement of rheumatoid factor by RAPA showed 14 patients with significant tiers (>1/160) at trial entry. Of these, 6 patients showed a fall of at least 2 titers on treatment with cA2, while the remaining patients showed a change of 1 titer or less. No patient showed a significant increase in RF titer during the trial. The median RF titer in the 11 patients fell from ½, 560 at entry to 1/480 by week 8 ($p>0.05$; Table 14). Specific RF isotypes were measured by ELISA, and showed falls in the 6 patients whose RAPA had declined significantly, as well as in some other patients. Median values for the three RF isotypes in the 14 patients seropositive at trial entry were 119, 102 and 62 IU/ml (IgM, IgG and IgA isotypes respectively) and at week 8 were 81, 64 and 46 IU/ml ($p>0.05$).

We tested sera from the first 9 patients for the presence of bioactive TNF, using the WEHI 164 clone 13 cytotoxicity assay (Espevik et al., *J. Imm. Methods* 95:99–105 (1986). In 8 patients, serum sets spanning the entire trial period were tested, while for patient 9, one pre-trial, one intermediate and the last available sample only were tested. The levels of bioactive TNF were below the limit of sensitivity of the assay in the presence of human serum (1 pg/ml). Since production of CRP and SAA are thought to be regulated in large part by IL-6, we also measured serum levels of this cytokine, using 2 different assays which measure total IL-6. In the Medgenix assay, IL-6 was significantly elevated in 17 of the 20 patients at entry. In this group, levels fell from 60 (18–500) pg/ml to 40 (0–230) pg/ml at week 1 ($p>0.05$; normal range<10 pg/ml) and to 32 (0–210) pg/ml at week 2 ($p<0.005$, $p<0.01$, adjusted). These results were supported by measurement of serum IL-6 in the first 16 patients in a separate ELISA developed in-house. IL-6 was detectable in 11 of the 16, with median (range) levels falling from 210 (25–900) pg/ml at entry to 32 (0–1,700) pg/ml at week 1 ($p<0.02$, $p<0.04$, adjusted; normal range<10 pg/ml) and to 44 (0–240) pg/ml at week 2 ($p<0.02$, $p<0.03$, adjusted).

We tested sera from the first 10 patients for the presence of anti-globulin responses to the infused chimeric antibody, but none were detected. In many patients however, cA2 was still detectable in serum samples taken at week 8 and this can have interfered with the ELISA.

Discussion

This is the first report describing the use of anti-TNFα antibodies in human autoimmune disease. Many cytokines are produced in rheumatoid synovium, but we chose to target specifically TNFα because of mounting evidence that it was a major molecular regulator in RA. The study results presented here support that view and allow three important conclusions to be drawn.

First, treatment with cA2 was safe and the infusion procedure was well tolerated. Although fever, headache, chills and hemodynamic disturbance have all been reported following treatment with anti CD4 or anti CDw52 in RA,

such features were absent in our patients. Also notable was the absence of any allergic event despite repeated treatment with the chimeric antibody, although the interval between initial and repeat infusions can have been too short to allow maximal expression of any anti-globulin response. The continuing presence of circulating cA2 at the conclusion of the trial amy have precluded detection of antiglobulin responses, but also implied that any such responses were likely to be of low titre and/or affinity. Although we recorded 2 infective episodes amongst the study group, these were minor and the clinical courses were unremarkable. TNFα has been implicated in the control of listeria and other infections in mice (Havell et al., *J. Immunol.* 143:2894–2899 (1989), but our limited experience does not suggest an increased risk of infections after TNFα blockade in man.

The second conclusion concerns the clinical efficacy of cA2. The patients we treated had long-standing, erosive, and for the most part seropositive disease, and had each failed therapy with several standard DMARDs. Despite this, the major clinical assessments of disease activity and outcome (morning stiffness, paid score, Ritchie index, swollen joint count and HAQ score) showed statistically significant improvement, even after adjustment for multiple comparisons. All patients graded their response as at least 'fair', with the majority grading it as 'good'. In addition, all achieved a response according to the criteria of Paulus and showed a mean improvement of at least 30% in 6 selected disease activity measures.

The improvements in clinical assessments following treatment with cA2 appear to be at least as good as those reported following treatment of similar patients with anti-leukocyte antibodies. The two therapeutic approaches can already be distinguished, however, by their effects on the acute phase response, since in several studies of anti-leukocyte antibodies, no consistent improvements in CRP or ESR were seen. In contrast, treatment with cA2 resulted in significant falls in serum CRP and SAA, with normalization of values in many patients. The changes were rapid and marked, and in the case of CRP, sustained for the duration of the study (Table 14). The falls in ESR were less marked, achieving statistical significance only when unadjusted (Table 14).

These results are consistent with current concepts that implicate TNFα in the regulation of hepatic acute phase protein synthesis, either directly, or by control of other, secondary, cytokines such as IL-6 (Fong et al., *J. Exp. Med.* 170:1627–1633 (1989), Guerne et al., *J. Clin. Invest.* 83:585–592 (1989)). In order to investigate the mechanism of control of the acute phase response in our patients, we measured serum TNFα and IL-6 before and after cA2 treatment. Bioactive TNFα was not detectable in baseline or subsequent sera. We used 2 different assays for IL-6, in view of previous reports of variability between different immunoassays in the measurement of cytokines in biological fluids (Roux-Lombard et al., *Clin. Exp. Rheum.* 10:515–520 (1992), and both demonstrated significant falls in serum IL-6 by week 2. These findings support the other objective laboratory changes induced by cA2, and provide in vivo evidence that TNFα is a regulatory cytokine for IL-6 in this disease. Amongst the other laboratory tests performed, rheumatoid factors fell significantly in 6 patients.

Neutralization of TNFα can have a number of beneficial consequences, including a reduction in the local release of cytokines such as IL-6 and other inflammatory mediators and modulation of synovial endothelial/leukocyte interactions. cA2 can also bind directly to synovial inflammatory cells expressing membrane TNFα, with subsequent in situ cell lysis.

The results obtained in this small series have important implications, both scientifically and clinically. At the scientific level, the ability of the neutralizing antibody, cA2, to reduce acute phase protein synthesis, reduce the production of other cytokines such as IL-6, and significantly improve the clinical state demonstrates that it is possible to interfere with the cytokine network in a useful manner without untoward effects. Due to the many functions and overlapping effects of cytokines such as IL-1 and TNFα, and the fact that cytokines induce the production of other cytokines and of themselves, there had been some pessimism as to whether targeting a single cytokine in vivo would have any beneficial effect (Kingsley et al., *Immunol. Today* 12:177–179 (1991), Trenthan, *Curr. Opin. Rheumatol.* 3:369–372 (1991)). This view is clearly refuted. On the clinical side, the results of short-term treatment with cA2 are significant and confirm that TNFα is useful as a new therapeutic target in RA.

EXAMPLE XXIII

TREATMENT WITH CHIMERIC ANTI-TNF IN A PATIENT WITH SEVER ULCERATIVE COLITIS

The patient is a 41 year old woman with long term ulcerative colitis, which was diagnosed by endoscopy and histology. She has a pancolitis, but the main disease activity was left-sided. There were no extra-intestinal complications in the past. Maintenance therapy consisted of Asacol(TM). Only one sever flair-up occurred 4 years previously and was successfully treated with steroids.

At beginning month one, she was admitted elsewhere because of a very sever flair-up of the ulcerative colitis. Treatment consisted of high doses of steroids intravenously, antibiotics, asacol and Total Parental Nutrition. Her clinical condition worsened and a colectomy was considered.

At end of month one, she was admitted at the internal ward of the AMC. Her main complaints consisted of abdominal pains, frequent water stools with blood and mucopus and malaise.

Medication: ASACOL 2 dd 500 mg, orally
Di-Adresone-T 1 dd 100 mg, intravenously
Flagyl 3 dd 500 mg, intravenously
Fortum 3 dd 1 gram, intravenously
Total parental nutrition via central venous catheter

On physical examination the patient was moderately ill with a weight of 55 kg and a temperature of 36° C. Jugular venous pressure was not elevated. Blood pressure was 110/70 mm Hg with a pulse rate of 80 per minute. No lymphadenopathy was found. Oropharynx was normal. Central venous catheter was inserted in situ with no signs of inflammation at the place of insertion. Normal auscultation of the lungs and heart. The abdomen was slightly distended and tender. Bowel sounds where reduced. Liver and spleen where not enlarged. No signs of peritonitis. Rectal examination was normal.

All cultures of the stools where negative.

Plain x-ray of the abdomen; slightly dilated colon. No thumb-printing, no free air, no toxic megacolon.

Sigmoidoscopy; (video-taped) Very severe inflammation with deep ulcers. Dilated rectum and sigmoid. Because of danger of perforation the color, the endoscopy was limited to the racto-sigmoid. No biopsies where taken.

Conclusion at time of admission: Severe steroid resistant flair-up of ulcerative colitis.

Antibiotics where stopped, because no improvement was noticed and there was no temperature.

After informed consent of the patient, treatment was started with 10 mg/kg bodyweight (a 550 mg) of cA2 chimeric monoclonal anti-TNF (Centocor) given intravenously over 2 hours (according the protocol of cA2 used in severe Crohn's disease).

During the infusion there were no complaints. Vital signs were monitored and were all normal. Before and after infusion blood samples where drawn. Two days after infusion she had less abdominal pain, the stool frequency decreased and no blood was seen in the stools any more. However she developed high temperature (40° C.). Blood-cultures where positive for *Staphylococcus epidermidis*. Infection of the central venous catheter was suspected. The catheter was removed and the same Staphylococcus was cultured from the tip of the central venous catheter. During this period she was treated with antibiotics for three days. After this her temperature dropped and she recovered substantially. Steroids were tapered off to 40 mg of prednisone daily.

After 14 days sigmoidoscopy was repeated and showed a remarkable improvement of the mucosa with signs of re-epithelization. There were no signs of bleeding, less mucous and even some normal vascular structures were seen.

At four months she was discharged.

At the outpatient clinic further monitoring was done weekly. Patient is still improving. Stool frequency is two times per day without blood or mucopus. Her laboratory improved, but there is still anaemia, probably due to iron deficiency. A colonoscopy is planned in the nearby future.

Our conclusion is that this patient had a very severe flair-up of her ulcerative colitis. She was refractory to treatment and a total colectomy was seriously considered. After infusion of cA2 the clinical course improved dramatically in spite of the fact that there was a complication of a sepsis which was caused by the central venous catheter.

EXAMPLE XXIV

p55 Fusion Protein Structure

The extracellular domains of the p55 and p75 receptors were expressed as Ig fusion proteins from DNA constructs designed to closely mimic the structure of naturally occurring, rearranged Ig genes. Thus, the fused genes included the promoter and leader peptide coding sequence of a highly expressed chimeric mouse-human antibody (cM-T412, Looney et all, *Hum. Antibody Hybridomas* 1992, 3, 191–200) on the 5' side of the TNF receptor insert, and codons for eight amino acids of human J sequence and a genomic fragment encoding all three constant domains of human IgG1 on the 3' side of the receptor insert position (FIGS. **27** and **28**).

Minor changes were introduced at the N-terminal ends of the heavy chain fusion proteins so that the first two amino acids would be identical or similar to the first two amino acids (Gln-Ile) encoded by the cM-T412 antibody gene (from which the leader peptide originated). This was done to increase the likelihood that any interactions between the N-terminal end of the mature protein and the leader peptide would still result in efficient transport into the lumen of the endoplasmic reticulum. Boyd et al., *Cell* 1990, 62, 1031–1033. Therefore, the Asp^1 and Ser^2 residues of naturally-occurring p55 were replaced with a Gln residue, and the Leu^1 residue of p75 was preceded by a Gln residue in all p75 constructs. No amino acid changes were introduced at the N-terminal end of the p55 light chain fusion.

Expression Vectors

PCR methodology was used to engineer cloned genes. Oligonucleotides were purchased from National Biosciences (Plymouth, Minn.). PCR amplification kits were from Perkin-Elmer (CA) and DNA sequencing kits from U.S. Biochemical Corporation (Cleveland, Ohio). Alkaline phosphatase-conjugated goat anti-human IgG was purchased from Jackson ImmunoResearch (West Grove, Pa.). ^{125}I-labeled human TNF was obtained from Du Pont Company, NEN (Boston, Mass.) and unlabeled recombinant human TNF from R&D Systems (Minneapolis, Minn.). Protein A-Sepharose beads was purchased from PHARMACIA (Piscataway, N.J.).

PCR methodology was used to engineer two cloned genes encoding the heavy chain or light chain of an efficiently expressed murine antibody, cM-T412 (see Looney et al.), for the purpose of directing the expression of foreign genes in a mammalian cell system. The approaches were to effectively delete the coding region of the antibody variable region and to place a unique restriction site in its place (StuI for the heavy chain vector and EcoRV for the light chain vector).

The resulting vector contained 2.5 kb of 5' flanking genomic DNA, the promoter, the leader peptide coding sequence (including the leader intron), a StuI cloning site to introduce inserts, coding sequence for eight amino acids of human J sequence Gly Thr Leu Val Thr Val Ser Ser (SEQ ID NO:6) followed by genomic sequences for the human IgG1 constant region. An analogous vector was made from the cM-T412 light chain gene except that an EcoRV cloning site was introduced at the carboxyl terminal end of the light chain leader peptide and a different human J sequence was encoded by the vector Gly Thr Lys Leu Glu Ile Lys (SEQ ID NO:7). Both vectors are based on plasmid pSV2-gpt and subsequent vector derivatives that contain genomic sequences for either the heavy chain or light chain constant regions. See Mulligan et al., *Science* 209:1422–1427 (1980). The *E. coli* gpt gene allows selection of transfected cells with mycophenolic acid.

Heavy Chain Vector A previously cloned EcoRI fragment containing the cM-T412 heavy chain gene (Goeddel et al., *Cold Spring Harbor Symp. Quant. Biol.* 1986, 51, 597–609) was subcloned into pUC19. This recombinant plasmid was used as a template for two PCR reactions. In one reaction, an oligo corresponding to the "reverse" primer of the pUC plasmids and the 3' oligo 5'-CCTGGATACCTGTGAAAAGA-3' (SEQ ID NO:8) (bold marks half of a StuI site; oligo was phosphorylated prior to the PCR reaction) were used to amplify a fragment containing 3 kb of 5' flanking DNA, the promoter, transcription start site and leader peptide coding sequence (including the leader intron). In the second reaction, the 5' oligo 5'-CCTGGTACCTTAGTCACCGTCTCCTCA-3' (SEQ ID NO:9) (bold marks half of a StuI site; oligo phosphorylated prior to the PCR reaction) and an oligo corresponding to the "forward" primer of pUC plasmids amplified a fragment encoding eight amino acids of human J sequence Gly Thr Leu Val Thr Val Ser Ser (SEQ ID NO:6) and a splice donor to allow splicing to the human constant region coding sequence provided in another vector. The two PCR fragments were digested with EcoRI and then simultaneously ligated into EcoRI-digested pUC19 to make pHC684 (FIG. **27**).

Because the StuI site formed at the junction of the two PCR fragments was followed by a 'GG' dinucleotide sequence, a dcm methylation site was formed preventing StuI from digesting that site when the DNA was grown in HB101 strain of *E coli*. Therefore, the plasmid DNA was

introduced into dcm-JM110 *E. coli* cells and reisolated. StuI was then able to cut at the junction but a second StuI site in the 5' flanking DNA was a apparent (DNA sequencing showed that StuI site to also he followed by a GG dinucleotide and therefore also methylated). To make the StuI cloning site at the junction be unique, a 790 bp XbaI fragment that included only one of the two StuI sites was subcloned into pUC19 to make the vector pHC707 (FIG. **27**A) which was then grown in JM110 cells. The StuI cloning site formed at the junction of the two PCR fragments second and third nucleotides (i.e., 'CA') of the last codon (Ala) of the signal sequence in order to maintain the appropriate translation reading frame (FIG. **27**).

A PCR fragment encoding a protein of interest can then be ligated into the unique StuI site of pHC707. The insert can include a translation stop codon that would result in expression of a "non-fusion" protein. Alternatively, a fusion protein could be expressed by the absence of a translation stop codon, thus allowing translation to proceed through additional coding sequences positioned downstream of the StuI cloning site. Coding sequences in the StuI site of pHC707 would not be fused directly to the IgG1 coding sequences in pHC730 but would be separated by an intron sequence that partially originates from pHC707 and partially from pHC730. These intron sequences would be deleted in the cell following transcription resulting in an RNA molecule that is translated into a chimeric protein with the protein of interest fused directly to the IgG1 constant domains.

The plasmid pHC730 was a modified form of an IgG1 expression, pSV2 gpt-hCyl vector described previously (Goeddel et al., *Cold Spring Harbor Symp. Quant. Biol.* 1986, 51, 597–609) (FIG. **28**). The modifications were (1) removal of the unique SalI and XbaI sites upstream of the constant region coding sequence, (2) insertion of a SalI linker into the unique BamHI site to allow use of SalI to linearize the plasmid prior to transfections, and (3) ligation into the unique EcoRI site the cloned cM-T412 EcoRI fragment but with the XbaI fragment flanking the V gene deleted (FIG. **29**). The resulting expression vector had a unique XbaI site for inserting the XbaI fragments from pHC707.

Light Chain Vector

A previously cloned HindIII fragment containing the cM-T412 light chain gene (Goeddel et al., *Cold Spring Harbor Symp. Quant. Biol.* 1986, 51, 597–609) was subcloned into pUC19 and the resulting plasmid used as template for PCR reactions. In one PCR reaction the "reverse" pUC primer and the 3' oligo 5'-AATAGATATCTCCTTCAACACCTGCAA-3' (SEQ ID NO:10) (EcoRV site is in bold) were used to amplify a 2.8 kb fragment containing 5' flanking DNA, the promoter, transcription start site and leader peptide coding sequence (including the leader intron) of the cloned light chain gene. This fragment was then digested with HindIII and EcoRV. In a second PCR reaction, the 5' oligo 5'-ATCGGGACAAAGTTGGAAATA-3' (SEQ ID NO:11) (bold marks half of an EcoRV site) and the "forward" pUC primer were used to amplify a fragment encoding seven amino acids of human J sequence (Gly Thr Lys Leu Glu Ile Lys) and an intron splice donor sequence. This fragment was digested with HindIII and ligated along with the other PCR fragment into pUC cut with HindIII. The resulting plasmid, pLC671 (FIG. **29**), has a unique EcoRV cloning site at the junction of the two PCR fragments with the EcoRV site positioned such that the first three nucleotides of the EcoRV site encoded the first amino acid of the mature protein (Asp).

The pLC671 HindIII insert was designed to be positioned upstream of coding sequences for the human kappa light chain constant region present in a previously described expression vector, pSV2gpt-hCk (FIG. **30**). However, pSV2gpt-hCk contained an EcoRV site it its gpt gene. Because it was desired that the EcoRV site in the pLC671 HindIII fragment be a unique cloning site after transferring the fragment into pSV2gpt-hCk, the EcoRV site in pSV2gpt-hCk was first destroyed by PCR mutagenesis. Advantage was taken of the uniqueness of this EcoRV site in pSV2gpt-hCk and a KpnI site 260 bp upstream of the EcoRV site. Therefore, the 260 bp KpnI-EcoRV fragment was removed from pSV2gpt-hCk and replaced with a PCR fragment that has identical DNA sequence to the 260 bp fragment except for a single nucleotide change that destroys the EcoRV site. The nucleotide change that was chosen was a T to a C in the third position of the EcoRV recognition sequence (i.e., GATATC changed to GACATC). Because the translation reading frame is such that GAT is a codon and because both GAT and GAG codons encode an Asp residue, the nucleotide change does not change the amino acid ended at that position. Specifically, pSV2gpt-hCk was used as template in a PCR reaction using the 5' oligo 5'-GGCGGTCTGGTACCGG-3' (SEQ ID NO:12) (KpnI site is in bold) and the 3' oligo 5'-GTCAACAACATAGTCATCA-3' (SEQ ID NO:13) (bold marks the complement of the ASP codon). The 260 bp PCR fragment was treated with the Klenow fragment of DNA polymerase to fill-in the DNA ends completely and then digested with KpnI. The fragment was ligated into pSV2gpt-hCk that had its KpnI-EcoRV fragment removed to make pLC327 (FIG. **30**).

The HindIII fragment of pLC671 was cloned into the unique HindIII site of pLC327 to make the light chain expression vector, pLC690 (FIG. **30**). This plasmid can be introduced into cells without further modifications to encode a truncated human kappa light chain, JCk, that contains the first two amino acids of the cM-T412 light chain gene, seven amino acids of human J sequences, and the light chain constant region. Alternatively, coding sequence of interest can be introduced into the unique EcoRV site of pLC690 to make a light chain fusion protein.

TNF Receptor DNA Constructs

For the p55 heavy chain fusion, amino acids 3–159 of the p55 extracellular domain were encoded in a PCR fragment generated using the 5' oligo 5'-CACAGGTGTGTCCCCAAGGAAAA-3' (SEQ ID NO:14) (bold marks the Val^3 codon) and the 3' oligo 5'-AATCTGGGGTAGGCACAA-3' (SEQ ID NO:15) (bold marks the complement of the Ile^{159} codon). For the p55 light chain fusion, amino acids 2–159 were encoded in a PCR fragment made Using the 5' oligo 5'-AGTGTGTGTCCCCAAGG-3' (SEQ ID NO:16) (bold marks the Ser^2 codon) and the same 3' oligo shown above. The light chain vector contained the codon for Asp^1 of p55. The DNA template for these PCR reactions was a previously reported human p55 cDNA clone. Gray et al., *Proc. Natl. Acad. Sci. USA* 1990, 87, 7380–7384.

A truncated light chain that lacked a variable region was expressed by transfecting cells with the light chain vector with no insert in the EcoRV cloning site. The resulting protein, termed JC_K, consisted of the first two amino acids of the cM-T412 light chain gene, seven amino acids of human J sequence (Gly Thr Lys Leu Glu Ile Lys) (SEQ ID NO:7), and the human light chain constant region.

A non-fusion form of p55 (p55-nf) was expressed in CHO-K1 cells using the CMV-major immediate early promoter after introducing a translation stop codon immediately after Ile^{159}. Secreted p55 was purified by affinity chromatography on a TNFα column.

Transfections and ELISA Assays

All plasmids were linearized with a restriction enzyme prior to introducing them into cells. Cells of the myeloma cell line X63-Ag8.653 were transfected with 12 μg of DNA by electroporation. Cell supernatants were assayed for IgG domains. Briefly, supernatants were incubated in plates coated with anti-human IgG Fc and then bound protein detected using alkaline phosphatase-conjugated anti-human and light chains.

Purification of Fusion Proteins

Cell supernatants were clarified by centrifugation followed by passage through a 0.45 micron filter. Supernatants were adjusted to 20 mM Tris-HCl, pH 8.3, 150 mM NaCl, and 1 mM EDTA (1×protein A buffer) and passed over a column of protein A-Sepharose beads. The column was washed in 1×protein A buffer followed by 100 mM Na Citrate, pH 5.0 to elute bound bovine IgG originating from the cell media. Bound fusion protein was then eluted in 100 mM Na Citrate, pH 3.5, neutralized with 0.2 volumes 1 M Tris, and dialyzed against PBS.

TNF Cytotoxicity Assays

TNF-sensitive WEHI-164 cells (Espevik et al., *J. Immunol. Methods* 1986, 95, 99–105) were plated in 1 μg/ml actinomycin D at 50,000 cells per well in 96-well microtiter plates for 3–4 hours. Cells were exposed to 40 pM TNFα or TNFβ and varying concentrations of fusion protein. The mixture was incubated overnight at 37° C. Cell viability was determined by adding 3-[4,5-dimethyl-thiazol-2-yl]-2, 5-diphenyltetrazolium bromide dye (MTT) to a final concentration of 0.5 mg/ml, incubating for 4 hours at 37° C., lysing the cells in 0.1 N HCl, 0.1% SDS and measuring the optical density at 550 nm wavelength.

Saturation Binding Analyses

Fusion proteins were captured while at a concentration of 10 ng/ml in 96-well microtiter plates coated with goat anti-human Fc antibodies. Varying concentrations of ^{125}I-TNF (34.8 μCi/μg) were added in PBS/1% BSA and allowed to bind for two hours at room temperature. Plates were washed and bound cpm determined. Non-specific binding was determined using an irrelevant antibody.

Several different versions of the p55 fusion proteins were expressed. Unlike what was reported for CD4 (Capon et al., *Nature* 1989, 337, 525–531) and IL-2 (Landolfi, *J. Biol. Chem.* 1991, 146, 915–919) fusion proteins that also included the C_H1 domain of the heavy chain, inclusion of a light chain proved to be necessary to get secretion of the Ig heavy chain fusion proteins from the murine myeloma cells. The light chain variable region was deleted to enable the TNF R domain on the heavy chain to bind TNF without stearic hindrance from the light chain.

The "double fusion" (df) protein, p55-df2, has p55 fused to both the heavy chain and light chain and is therefore tetravalent with regard to p55. p55-sf3 has the p55 receptor (and the same eight amino acids of human J sequence present in p55-sf2 and p55-df2) linked to the hinge region, i.e., the C_H1 domain of the constant region is deleted.

After one or two rounds of subcloning, spent cell supernatant from the various cell lines were yielding 20 μg/ml (for p55-sf2) of fusion protein. The proteins were purified from the spent supernatant by protein A column chromatography and analyzed by SDS-PAGE with or without a reducing agent. Each fusion protein was clearly dimeric in that their M_r estimates from their migration through a non-reducing gel was approximately double the estimated M_r from a reducing gel. However, two bands were seen for p55-sf2 and p55-df2. Two lines of evidence indicated that, in each case, the lower bands did not include a light chain while the upper bands did include a light chain. First, when p55-sf2 containing both bands were passed over an anti-kappa column, the upper band bound to the column while the lower band passed through the column. Second, Western blots have shown that only the upper bands were reactive with anti-kappa antibodies.

It is believed that the versions of these fusion proteins that do not have a light chain (k) were not secreted to a significant degree but rather were primarily released from dead cells because 1) supernatants from cells transfected with the p55 heavy chain fusion gene and no light chain gene did not have detectable fusion protein until after there was significant cell death, and 2) the ratio of the k− to k+ versions of p55-sf2 increased as cell cultures went from 95% viability to 10% viability.

WEHI Cytotoxicity Assays

The ability of the various fusion proteins to bind and neutralize human TNFα or TNFβ was tested in a TNF-mediated cell killing assay. Overnight incubation of the murine fibrosarcoma cell line, WEHI 164 (Espevik et al., *J. Immunol. Methods* 1986, 95, 99–105), with 20 pM (1 ng/ml) TNFα results in essentially complete death of the culture. When the fusion proteins were pre-incubated with TNFα (FIG. **31**A) or TNFβ (FIG. **31**B and Table 1 above) and the mixture added to cells, each fusion protein demonstrated dose-dependent protection of the cells from TNF cytotoxicity. Comparison of the viability of control cells not exposed to TNF to cells incubated in both TNF and fusion protein showed that the protection was essentially complete at higher concentrations of fusion protein.

Tetravalent p55-df2 showed the greatest affinity for TNFα requiring a concentration of only 55 pM to confer 50% inhibition of 39 pM (2 ng/ml) TNFα (FIG. **31**A and Table 1). Bivalent p55-sf2 and p75P-sf2 were nearly as efficient, requiring concentrations of 70 pM to half-inhibit TNFα. Approximately two times as much p75-sf2 was required to confer 50% inhibition compared to p55-sf2 at the TNF concentration that was used. The monomeric, non-fusion form of p55 was much less efficient at inhibiting TNFα requiring a 900-fold molar excess over TNFα to inhibit cytotoxicity by 50%. This much-reduced inhibition was also observed with a monomeric, Fab-like p55 fusion protein that was required at a 2000-fold molar excess over TNFα to get 50% inhibition. The order of decreasing inhibitory activity was therefore p55-df2>p55-sf2=p75P-sf2>p75-sf2>>>monomeric p55.

EXAMPLE XXV p75

To make a p75 heavy chain fusion (p75-sf2), amino acids 1–235 (Smith et al., *Science* 1990, 248, 1019–1023 and Kohno et al., *Proc. Natl. Acad. Sci.* 1990, 87, 8331–8335) were encoded in a fragment prepared using the 5' oligo 5'-CACAGCTGCCCGCCCAGGTGGCAT-3' (SEQ ID NO:17) (bold marks the Leu^1 codon) and the 3' oligo 5'-GTCGCCAGTGCTCCCTT-3' (SEQ ID NO:18) (bold marks the complement of the Asp^{235} codon). Two other p75 heavy chain fusions (p75P-sf2 and p75P-sf3) were made using the same 5' oligo with the 3' oligo 5'-ATCGGACGTGGACGTGCAGA-3' (SEQ ID NO:19). The resulting PCR fragment encoded amino acids 1–182. The PCR fragments were blunt-end ligated into the StuI or EcoRV site of the appropriate vector and checked for the absence of errors by sequencing the inserts completely.

Several different versions of the p75 fusion proteins were also expressed. p75-sf2 has the complete extracellular

domain of p75 fused to the heavy chain while p75P-sf2 lacks the C-terminal 53 amino acids of the p75 extracellular domain. p75P-sf3 is the same as p75P-sf2 except that it lacks the C_H1 domain. The region deleted in p75P-sf2 and -sf3 contains sites of O-linked glycosylation and a proline-rich region, neither of which is present in the extracellular domain of p55. Seckinger et al., *Proc. Nat. Acad. Sci. USA* 1990, 87, 5188–5192.

Similar to p55-sf2, two bands were seen for p75-sf2 and p75P-sf2 (FIG. **32**B, lane **8**).

Surprisingly, the order of decreasing inhibitory activity was different for TNFβ, as presented in FIG. **32**. p75P-sf2 was most efficient at inhibition requiring a concentration of 31 pM to half-inhibit human TNFβ at 2 pM. Compared to p75P-sf2, three times as much p75-sf2 and three times as much p55-sf2 were necessary to obtain the same degree of inhibition. The order of decreasing inhibitory activity was therefore p75P-sf2>p75-sf2=p55-sf2.

Affinity Measurements

A comparison was made of the binding affinity of various fusion proteins and TNFα by saturation binding (FIGS. **33**A and **33**B) and Scatchard analysis (FIGS. **33**C–H). A microtiter plate was coated with excess goat anti-Fc polyclonal antibody and incubated with 10 ng/ml of fusion protein in TBST buffer (10 mM Tris-HCl, pH 7.8, 150 NaCl, 0.05% Tween-20) for 1 hour. Varying amounts of ^{125}I labeled TNFα (specific activity—34.8 μCi/μg) was then incubated with the captured fusion protein in PBS (10 mM Na Phosphate, pH 7.0, 150 mM NaCl) with 1% bovine serum albumin for 2 hours. Unbound TNFα was washed away with four washes in PBS and the cpm bound was quantitated using a γ-counter. All samples were analyzed in triplicate. The slope of the lines in (FIGS. **33**C–H) represent the affinity constant, K_a. The dissociation constant (K_d) values (see Table 1) were derived using the equation $K_d=1/K$.

EXAMPLE XXVI

In vivo Results

C3H mice were challenged with 5 μg of human TNFα after treatment with an immunoreceptor molecule of the invention. The effect of the treatment was compared with two control treatments. The first control, cA2, is a chimeric mouse/human IgG_1 monoclonal antibody that binds human TNF, and thus is a positive control. The second control, c17-1A, is a chimeric mouse/human IgG_1 irrelevant monoclonal antibody and is thus a negative control. The results of the treatments were as presented in the following Table 17.

TABLE 17

Treatment	Dead Fraction	% Dead
1 μg cA2	5/14	36%
10 μg CA2	1/15	7%
50 μg c17-1A	13/15	87%
1 μg p55-sf2	8/15	53%
10 μg p55-sf2	0/15	0%
50 μg p55-sf2	0/15	0%

Mice were injected with 25 μg of p55 fusion protein or a control antibody and 1 hour later were challenged with 1 μg lipopolysaccharide (type J5). Mice were checked 24 hours later. The results are presented in the following Table 18.

TABLE 18

Treatment	Dead Fraction	% Dead
Control Antibody	11/11	100%
p55-sf2	0/13	0%

All references cited herein, including journal articles or abstracts, published or corresponding U.S. or foreign patent applications, issued U.S. or foreign patents, or any other references, are entirely incorporated by reference herein, including all data, tables, figures, and text presented in the cited references. Additionally, the entire contents of the references cited within the references cited herein are also entirely incorporated by reference.

Reference to known method steps, conventional methods steps, known methods or conventional methods is not in any way an admission that any aspect, description or embodiment of the present invention is disclosed, taught or suggested in the relevant art.

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art (including the contents of the references cited herein), readily modify and/or adapt for various applications such specific embodiments, without undue experimentation, without departing from the general concept of the present invention. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed embodiments, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance presented herein, in combination with the knowledge of one of ordinary skill in the art.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(iii) NUMBER OF SEQUENCES: 19

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 157 amino acids

-continued

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Val Arg Ser Ser Ser Arg Thr Pro Ser Asp Lys Pro Val Ala His Val
 1               5                  10                  15

Val Ala Asn Pro Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg
            20                  25                  30

Ala Asn Ala Leu Leu Ala Asn Gly Val Glu Leu Arg Asp Asn Gln Leu
        35                  40                  45

Val Val Pro Ser Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe
    50                  55                  60

Lys Gly Gln Gly Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile
65                  70                  75                  80

Ser Arg Ile Ala Val Ser Tyr Gln Thr Lys Val Asn Leu Leu Ser Ala
                85                  90                  95

Ile Lys Ser Pro Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys
            100                 105                 110

Pro Trp Tyr Glu Pro Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu Lys
        115                 120                 125

Gly Asp Arg Leu Ser Ala Glu Ile Asn Arg Pro Asp Tyr Leu Asp Phe
    130                 135                 140

Ala Glu Ser Gly Gln Val Tyr Phe Gly Ile Ile Ala Leu
145                 150                 155
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 321 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..321

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
GAC ATC TTG CTG ACT CAG TCT CCA GCC ATC CTG TCT GTG AGT CCA GGA      48
Asp Ile Leu Leu Thr Gln Ser Pro Ala Ile Leu Ser Val Ser Pro Gly
  1               5                  10                  15

GAA AGA GTC AGT TTC TCC TGC AGG GCC AGT CAG TTC GTT GGC TCA AGC      96
Glu Arg Val Ser Phe Ser Cys Arg Ala Ser Gln Phe Val Gly Ser Ser
            20                  25                  30

ATC CAC TGG TAT CAG CAA AGA ACA AAT GGT TCT CCA AGG CTT CTC ATA     144
Ile His Trp Tyr Gln Gln Arg Thr Asn Gly Ser Pro Arg Leu Leu Ile
        35                  40                  45

AAG TAT GCT TCT GAG TCT ATG TCT GGG ATC CCT TCC AGG TTT AGT GGC     192
Lys Tyr Ala Ser Glu Ser Met Ser Gly Ile Pro Ser Arg Phe Ser Gly
    50                  55                  60

AGT GGA TCA GGG ACA GAT TTT ACT CTT AGC ATC AAC ACT GTG GAG TCT     240
Ser Gly Ser Gly Thr Asp Phe Thr Leu Ser Ile Asn Thr Val Glu Ser
65                  70                  75                  80

GAA GAT ATT GCA GAT TAT TAC TGT CAA CAA AGT CAT AGC TGG CCA TTC     288
Glu Asp Ile Ala Asp Tyr Tyr Cys Gln Gln Ser His Ser Trp Pro Phe
                85                  90                  95

ACG TTC GGC TCG GGG ACA AAT TTG GAA GTA AAA                        321
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-continued

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Thr Phe Gly Ser Gly Thr Asn Leu Glu Val Lys
            100                 105


(2) INFORMATION FOR SEQ ID NO:3:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 107 amino acids
          (B) TYPE: amino acid
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Asp Ile Leu Leu Thr Gln Ser Pro Ala Ile Leu Ser Val Ser Pro Gly
  1               5                  10                  15

Glu Arg Val Ser Phe Ser Cys Arg Ala Ser Gln Phe Val Gly Ser Ser
             20                  25                  30

Ile His Trp Tyr Gln Gln Arg Thr Asn Gly Ser Pro Arg Leu Leu Ile
         35                  40                  45

Lys Tyr Ala Ser Glu Ser Met Ser Gly Ile Pro Ser Arg Phe Ser Gly
     50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Ser Ile Asn Thr Val Glu Ser
 65                  70                  75                  80

Glu Asp Ile Ala Asp Tyr Tyr Cys Gln Gln Ser His Ser Trp Pro Phe
                85                  90                  95

Thr Phe Gly Ser Gly Thr Asn Leu Glu Val Lys
            100                 105


(2) INFORMATION FOR SEQ ID NO:4:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 357 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (ix) FEATURE:
          (A) NAME/KEY: CDS
          (B) LOCATION: 1..357

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GAA GTG AAG CTT GAG GAG TCT GGA GGA GGC TTG GTG CAA CCT GGA GGA      48
Glu Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
  1               5                  10                  15

TCC ATG AAA CTC TCC TGT GTT GCC TCT GGA TTC ATT TTC AGT AAC CAC      96
Ser Met Lys Leu Ser Cys Val Ala Ser Gly Phe Ile Phe Ser Asn His
             20                  25                  30

TGG ATG AAC TGG GTC CGC CAG TCT CCA GAG AAG GGG CTT GAG TGG GTT     144
Trp Met Asn Trp Val Arg Gln Ser Pro Glu Lys Gly Leu Glu Trp Val
         35                  40                  45

GCT GAA ATT AGA TCA AAA TCT ATT AAT TCT GCA ACA CAT TAT GCG GAG     192
Ala Glu Ile Arg Ser Lys Ser Ile Asn Ser Ala Thr His Tyr Ala Glu
     50                  55                  60

TCT GTG AAA GGG AGG TTC ACC ATC TCA AGA GAT GAT TCC AAA AGT GCT     240
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Ser Ala
 65                  70                  75                  80

GTC TAC CTG CAA ATG ACC GAC TTA AGA ACT GAA GAC ACT GGC GTT TAT     288
Val Tyr Leu Gln Met Thr Asp Leu Arg Thr Glu Asp Thr Gly Val Tyr
                85                  90                  95

TAC TGT TCC AGG AAT TAC TAC GGT AGT ACC TAC GAC TAC TGG GGC CAA     336
Tyr Cys Ser Arg Asn Tyr Tyr Gly Ser Thr Tyr Asp Tyr Trp Gly Gln
```

-continued

```
          100                 105                 110

GGC ACC ACT CTC ACA GTC TCC                                    357
Gly Thr Thr Leu Thr Val Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Glu Val Lys Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
  1               5                  10                  15

Ser Met Lys Leu Ser Cys Val Ala Ser Gly Phe Ile Phe Ser Asn His
             20                  25                  30

Trp Met Asn Trp Val Arg Gln Ser Pro Glu Lys Gly Leu Glu Trp Val
         35                  40                  45

Ala Glu Ile Arg Ser Lys Ser Ile Asn Ser Ala Thr His Tyr Ala Glu
     50                  55                  60

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Ser Ala
 65                  70                  75                  80

Val Tyr Leu Gln Met Thr Asp Leu Arg Thr Glu Asp Thr Gly Val Tyr
                 85                  90                  95

Tyr Cys Ser Arg Asn Tyr Tyr Gly Ser Thr Tyr Asp Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Thr Leu Thr Val Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Gly Thr Leu Val Thr Val Ser Ser
  1               5
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Gly Thr Lys Leu Glu Ile Lys
  1               5
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

-continued

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CCTGGATACCTGTGAAAAGA 20

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CCTGGTACCTTAGTCACCGTCTCCTCA 27

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

AATAGATATCTCCTTCAACACCTGCAA 27

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ATCGGGACAAAGTTGGAAATA 21

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GGCGGTCTGGTACCGG 16

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

-continued

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GTCAACAACATAGTCATCA 19

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CACAGGTGTGTCCCCAAGGAAAA 23

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

AATCTGGGGTAGGCACAA 18

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

AGTGTGTGTCCCCAAGG 17

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CACAGCTGCCCGCCCAGGTGGCAT 24

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GTCGCCAGTGCTCCCTT 17

-continued

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

ATCGGACGTGGACGTGCAGA 20

What is claimed is:

1. A chimeric antibody comprising at least part of a human immunoglobulin constant region and at least part of a non-human immunoglobulin variable region, said antibody capable of binding an epitope specific for human tumor necrosis factor TNFα, wherein the non-human immunoglobulin variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 3 and SEQ ID NO: 5.

2. An immunoassay method for detecting human TNF in a sample, comprising:

(a) contacting said sample with an antibody according to claim **1**, or a TNF binding fragment thereof, in detectably labeled form; and

(b) detecting the binding of the antibody to said TNF.

3. A chimeric antibody comprising at least part of a human immunoglobulin constant region and at least part of a non-human immunoglobulin variable region, said antibody capable of binding an epitope specific for human tumor necrosis factor TNFα, wherein the non-human immunoglobulin variable region comprises a polypeptide encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 2 and SEQ ID NO: 4.

4. An immunoassay method for detecting human TNF in a sample, comprising:

(a) contacting said sample with an antibody according to claim **3**, or a TNF binding fragment thereof, in detectably labeled form; and

(b) detecting the binding of the antibody to said TNF.

5. A chimeric antibody, comprising two light chains and two heavy chains, each of said chains comprising at least part of a human immunoglobulin constant region and at least part of a non-human immunoglobulin variable region, said variable region capable of binding an epitope of human tumor necrosis factor hTNFα, wherein said light chains comprise variable regions comprising SEQ ID NO: 3 and said heavy chains comprise variable regions comprising SEQ ID NO: 5.

6. A chimeric antibody according to claim **5**, wherein the human immunoglobulin constant region is an IgG1.

7. A chimeric antibody comprising at least part of a human IgG1 constant region and at least part of a non-human immunoglobulin variable region, said antibody capable of binding an epitope specific for human TNFα, wherein the non-human immunoglobulin variable region comprises a polypeptide encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 2 and SEQ ID NO: 4.

8. A polypeptide comprising the amino acid sequence of SEQ ID NO: 3, wherein said polypeptide binds to hTNFα and competitively inhibits the binding of monoclonal antibody cA2 to hTNFα.

9. A polypeptide comprising the amino acid sequence of SEQ ID NO: 5, wherein said polypeptide binds to hTNFα and competitively inhibits the binding of monoclonal antibody cA2 to hTNFα.

* * * * *

UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF MASSACHUSETTS

JANSSEN BIOTECH, INC., and NEW YORK UNIVERSITY, Plaintiffs,	)	
v.	)	Civil Action No. 15-CV-10698-MLW
CELLTRION HEALTHCARE CO., LTD, CELLTRION, INC., and HOSPIRA, INC., Defendants.	)	

BEFORE THE HONORABLE MARK L. WOLF
UNITED STATES DISTRICT JUDGE

MOTION HEARING

August 17, 2016
1:05 p.m.

John J. Moakley United States Courthouse
Courtroom No. 10
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P R O C E E D I N G S

THE COURT: Good afternoon. Would counsel please identify themselves for the Court and for the record.

MR. DISKANT: Greg Diskant for the plaintiffs, along with Irena Royzman, Barbara Mullin. We've got a substitute back here, Nathan Monroe-Yavneh, and our tech guy.

THE COURT: Okay.

MR. HURST: Jim Hurst on behalf of defendants. Client representatives are here as well, Jeff Myers and David Kim. And I'll let the others introduce themselves.

MS. CUTRI: Good morning, Your Honor. Elizabeth Cutri of Kirkland & Ellis.

MR. KLEIN: Good morning, Your Honor. Chuck Klein of Winston & Strawn.

MR. HOANG: Good afternoon. Dan Hoang, Winston Strawn.

MS. MARTIN: Good afternoon, Your Honor. Andrea Martin from Burns & Levinson.

MR. KELLY: Good afternoon, Your Honor. Dennis Kelly Burns & Levinson.

THE COURT: Okay. I have not heard from you since we recessed yesterday. So I assume there's -- maybe the two people in the front row there would go out of the courtroom. Right there, the two who were just talking. I can't do this. I've got a dozen lawyers here. You're welcome to talk in the

hallway. You're not welcome to talk in the courtroom. You're sitting right in front of me. It's extremely distracting. You can stay if you'll heed that admonition, please.

I'm sorry. Counsel were rising.

MR. HURST: Discussions did occur. I know both parties were doing their best to see if they could reach some common agreement here, and it was, unfortunately, unsuccessful. That's my understanding, Your Honor.

MR. DISKANT: That's my understanding as well, Your
01:06 Honor. I know the parties are committed to continuing to talk to each other, and I'm sure they will do that.

THE COURT: Okay. I am prepared to give you my decision on the '471 patent obviousness double patenting motion for summary judgment. I'm not yet prepared to give you my decision with regard to the reexamination motion. I expect I'll do that tomorrow. After I explain my reasoning for allowing the defendants' motion based on obviousness double patenting, we'll move to the argument on the '083 patent.

All right. I'm deciding this matter orally, as I said
01:08 yesterday. There's urgency to it under this new statutory regime for biosimilar drugs. I am immersed in this and have your arguments vividly in mind. Although I'm deciding this orally, I hope it will be evident that I'm not deciding it casually. The transcript will be the immediate record of the decision. I may, very well may convert the transcript at some

point into a more formal memorandum and order.

As I said, the defendants' motion for summary judgment on the '471 patent based on obviousness double patenting, docket number 127, is hereby allowed. The parties agree that no material facts are in dispute. The issue is solely a question of law. Therefore, this issue is ripe for resolution on the defendants' motion for summary judgment.

With regard to background, the plaintiffs, Janssen Biotech, Inc. and New York University, who are sometimes collectively called Janssen, are the holders of patents related to a biologic medication called Remicade, which is based on an antibody called infliximab.

Plaintiffs allege that defendants Celltrion and Hospira have infringed these patents by filing an abbreviated Biologic License Application for a product that is "biosimilar" to Remicade. Two patents are at issue in this motion for summary judgment based on obviousness-type double patenting. They are U.S. Patent Number 6,284,471, which I'll refer to as the '471 patent or the '471, and U.S. Patent Number 6,790,444, to which I will refer as the '444 patent or the '444. The '471 patent covers a genus, or group, of compounds that includes infliximab. The '444 patent is for the infliximab antibody specifically.

Plaintiffs concede that the '444 patent claims are not patentably distinct from the '471 patent claims. Both patents

are based on an application filed in 1991, which is sometimes called the "priority application." The priority date for each patent is 1991.

The '471 patent was filed in 1994 and issued on September 4, 2001. If it stood alone, it would expire on September 4, 2018 because it was filed before the 1995 effective date of the law altering patent terms, the Uruguay Round Agreements Act, or URAA, which the defendant at least sometimes called the GATT.

01:12 The statute is codified at 35 U.S.C. Section 154, most pertinently at Section 154(c)(1). The URAA provides protection for 20 years from the date of the original, priority application or 17 years after issuance, whichever is longer, for applications filed before 1995. Therefore, if the '471 patent stood alone, it would expire in 2018. However, for applications filed after 1995, patent protection extends for 20 years after the date the original, priority application was filed. The application for the '444 patent was filed in 2001, after the 1995 effective date of the URAA, and was issued in 01:12 2004. As it was based on a 1991 priority application, it expired 20 years later, in 2011.

In what the parties call the defendants' *Gilead* motion, the defendants seek summary judgment of invalidity on Claims 1, 3, 5, 6 and 7 of the '471 patent for obviousness-type double patenting based on the '444 patent. The only question

presented by the motion is whether, in view of the Federal Circuit's decision in *Gilead Sciences, Inc. v. Natco Pharma Limited,* 753 F.3d 1278, a 2014 decision, the earlier-expiring '444 patent should be held to be a double patenting reference that invalidates the '471 patent. I find that it is such a reference, and therefore the '471 patent is invalid.

Gilead involved two patents based on applications filed after 1995. Therefore, it did not implicate the provision of the URAA that provides patent protection for at least 17 years after issuance if the application for a patent at issue was filed before 1995. As this case is factually different than *Gilead*, *Gilead* is not binding precedent for the purpose of deciding this case. I find, however, that in enacting the URAA, Congress and the President did not intend to alter the judicially-created doctrine of obviousness double patenting or restrict the power of the courts to apply it to patents resulting from applications filed before 1995.

I also find that the Federal Circuit would apply the *Gilead* ruling to the circumstances of this case and again find that a later-issued but earlier-expiring patent can serve as a reference that renders an earlier-issued but later-expiring patent invalid for obviousness double patenting. In *Gilead*, 253 F.3d at 1214, the Federal Circuit wrote, "It is a bedrock principle of our patent system that when a patent expires, the public is free to use not only the same invention claimed in

the expired patent but also obvious or patentably indistinct modifications of that invention." It then cites *In re Longi* for the proposition that the public should be able to act on the assumption that upon the expiration of a patent, it will be free to use not only the invention claimed in the patent but also any modifications or variants thereof which would have been obvious to those of ordinary skill in the art at the time the invention was made.

"The double patenting doctrine has always been implemented to effectively uphold that principle," said the Federal Circuit. The plaintiff acknowledges that the invention claimed in the '471 patent is obvious or patentably indistinct -- I'm sorry -- is an obvious or patentably indistinct modification of the invention claimed in the '444 patent.

In *Gilead* at 1210, the Federal Circuit explained that the obviousness-type double patenting doctrine prohibits an inventor from extending his right to exclude through claims in a later-expiring patent that are not patentably distinct from claims of the inventor's earlier-expiring patent. The Federal Circuit noted at page 1212 that Federal Courts had applied this principle for over a century.

In *Gilead*, at 1216, the Federal Circuit essentially rejected plaintiffs' argument here that the URAA manifests a statutory intent to provide patents emerging from applications

filed before 1995 with at least 17 years' protection despite the otherwise applicable judicial doctrine of obviousness double patenting. The URAA is silent on this issue. It does not state that pre-URAA patents will always have 17 years' protection, nor does it reference the doctrine of obviousness double patenting.

Generally, the Supreme Court "presumes that legislatures act with case law in mind," as the Supreme Court wrote in *Abuelhawa v. United States*, 129 Supreme Court 2102 at 2106, a 2009 decision. The Supreme Court made a similar statement in *Miles v. Apex*, 111 Supreme Court 317 at 325. Consistent with this well-established canon, the Federal Circuit wrote in *Gilead* at 1216, "Congress could not have intended to inject the potential to disturb the consistent application of the doctrine of double patenting by passing the URAA."

In *Gilead* at 1215, the Federal Circuit stated that, "The primary ill avoided by enforcement of the double patenting doctrine is a restriction on the public's freedom to use the invention claimed in a patent and all obvious modifications after that patent expired." Therefore, the Federal Circuit held, at 1217, that an earlier-expiring patent can qualify as an obviousness-type double patenting reference for a later-expiring patent under the circumstances here. In reaching this conclusion in reversing the decision of the

District Court, the Federal Circuit at 1211 stated that the District Court had mistakenly relied on the reasoning of two pre-*Gilead* decisions involving, as this case does, pre- and post-URAA patents. There are two cases on which the plaintiff relies here. *Abbott Labs*, 2011 Westlaw 1897322, a 2011 Delaware decision, and *Brigham and Women's Hospital*, 761 F. Supp. 2d 210, another 2011 District of Delaware decision.

In *Gilead*, the Court noted at 1211 in footnote 2, that in *Ex-Parte Pfizer*, the Board of Patent Appeals, on facts analogous to the facts of the instant case, found that the later-issued but earlier-expiring patent invalidated an earlier-issued later-expiring patent under the doctrine of obviousness double patenting because the later-expiring patent, in the Board's opinion, would impermissibly block the public from practicing the invention and obvious derivations thereof disclosed in the patents that expired first.

This reference to *Pfizer*, among all of the reasoning in *Gilead*, indicates to me that the Federal Circuit would in this case find the '471 patent obvious and invalid in view of the expired '444 patent. If the plaintiffs' position were correct, the public would be prevented from practicing the expired '444 patent and an obvious patentably indistinct variation of it. This would violate the bedrock principle that when a patent expires, the public is free to use the invention claimed and obvious variations of it, which is at the heart of

the obviousness double patenting doctrine, which the Federal Circuit has found to be unaltered by the URAA.

The obviousness double patenting document was well established when plaintiff applied for and accepted the '444 patent, which it knew would expire in 2011. The plaintiff decided to take at least the risk that the '471 would be deemed invalid when the '444 expired.

Infliximab was covered by the '471 genus patent, which plaintiff obtained, and by the '444 specious patent that specifically claimed that antibody. As plaintiffs' counsel acknowledged at the August 16, 2016 hearing, such narrower patents are generally acquired to protect against claims of invalidity or infringement. That risk was real here as the PTO has, in the pending reexamination, found that the '471 is obvious and invalid in view of two other patents plaintiffs held, the '195 and the '272.

Although not material to the analysis, I note that the plaintiff had a significant incentive to try to avoid the risk of invalidity of the '471 patent by obtaining the '444 patent as the parties have agreed in informing the court that Remicade has generated sales of more than $4 billion a year.

In *AbbVie*, 764 F.3d 1366 at 1374, a 2014 Federal Circuit case, the Federal Circuit confirmed that the doctrine of obviousness-type double patenting continues to apply where two patents that claim the same invention have different

expiration dates. It reiterated the ruling of *Gilead* at page 1379 of *AbbVie*, that if the later-expiring patent is merely an obvious variation of the invention disclosed and claimed in the reference patent, the later-expiring patent is invalid for obviousness-type double patenting.

I find that this reasoning is equally applicable to the facts of this case. More specifically, I hold that the expired '444 patent is a reference for the '471 patent. The '471 patent is not patentably distinct from the '444 patent, therefore the '471 patent is invalid.

I note that this conclusion is consistent with what is evidently the only other decision on comparable facts. *MLC Intellectual Property*, 2016 Westlaw, 41920009, a Northern District of California case, decided on August 9, 2006, in addressing the issues presented here, briefly on page 3 in note 4.

Accordingly, as I said, the defendants' motion for summary judgment finding the '471 patent invalid, docket number 127, is hereby allowed.

So have the parties discussed who should go first with regard to the claim construction of the '083?

MR. DISKANT: Yes, Your Honor. We agreed that, since we are the plaintiffs, we would go first on claim construction. And my colleague Irena Royzman will present our argument.

THE COURT: Okay.

UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF MASSACHUSETTS

JANSSEN BIOTECH, INC., and NEW YORK UNIVERSITY, Plaintiffs,	)	
v.	)	Civil Action No. 15-CV-10698-MLW
CELLTRION HEALTHCARE CO., LTD, CELLTRION, INC., and HOSPIRA, INC., Defendants.	)	

BEFORE THE HONORABLE MARK L. WOLF
UNITED STATES DISTRICT JUDGE

MOTION HEARING

August 18, 2016
1:04 p.m.

John J. Moakley United States Courthouse
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P R O C E E D I N G S

THE COURT: Would counsel please identify themselves for the record.

MR. DISKANT: Good afternoon, Your Honor. Greg Diskant on behalf of Janssen, with my colleagues, Irena Royzman, Barbara Mullin and Jamison Davies.

MR. HURST: Jim Hurst on behalf the defendants. Representative here for Pfizer Hospira is Jeff Myers. And I'll let counsel identify themselves.

01:04 MS. CUTRI: Good afternoon, Your Honor. Elizabeth Cutri of Kirkland & and Ellis.

MR. KLEIN: Good afternoon, Your Honor. Chuck Klein of Winston & Strawn.

MR. HOANG: Good afternoon, Your Honor. Dan Hoang for Winston & Strawn.

MS. MARTIN: Good afternoon, Your Honor. Andrea Martin from Burns & Levinson.

MR. KELLY: Good afternoon, Your Honor. Dennis Kelly from Burns & Levinson.

01:05 THE COURT: Okay. I have on the agenda today --

MR. DISKANT: Excuse me, Your Honor. I'm sorry, I neglected to mention that our local counsel, Heather Repicky, is also here.

THE COURT: Okay. I have on the agenda for today to give you my ruling on the motion for summary judgment on the

reexamination issue concerning the '471, the motion for an expedited trial, the stipulation concerning consolidation, the possible Rule 54(b) motion and perhaps scheduling a pretrial conference.

Is there anything else that should be on the agenda?

MR. DISKANT: I don't think so, Your Honor.

MR. HURST: I believe that covers it, Your Honor.

THE COURT: Okay. All right.

As you know, yesterday I granted the defendants' motion for summary judgment on the '471 patent that the parties have been referring to as the *Gilead* motion. There's a second motion for summary judgment on the '471 patent. That is the defendants' motion for summary judgment on invalidity of claims 1, 3, 5, 6, 7 of the '471 patent for obviousness-type double patenting in light of the claims in the '195 and '272 patents. That is docket number 176.

In the interest of completeness, given the urgency and consequential nature of this case, I think it's prudent to decide both motions for summary judgment. For the reasons I'll explain, this pending motion, 176, is also meritorious and is being allowed.

In summary, as I'll explain, I find that plaintiffs, Janssen Biotech, Inc. and New York University's '471 patent is not protected by the safe harbor provided by 35 United States Code Section 121. I also find that the one-way test for

obviousness double patenting applies in this case. And as the plaintiffs concede, for present purposes, the claims in the '471 are obvious in view of the earlier-issued claims in the '195 and '272.

In addition, assuming without finding that the two-way obviousness test could apply, depending on the facts, there may be disputed facts concerning whether the Patent and Trademark Office, PTO, was solely responsible for any delay that caused the '195 and '272 to issue before the '471. However, any such genuine disputes would not be material because applying the two-way test in this case would properly involve consideration of the specification of the '471. And in view of the specification, the claims in the '195 and '272 are obvious in view of the claims in the '471.

The following undisputed facts are among those that are relevant:

On February 2, 1993, plaintiffs filed U.S. Patent Application number 8/013413, known as the '413 application, which is a parent application to the '471, the '195 and '272 patents. During prosecution of the '413 application, the PTO examiner issued a restriction requirement that, among other things, required plaintiffs to separate claims to the chimeric antibodies and those relating to methods for using antibodies to treat various conditions into separate applications.

On February 4, 1994, plaintiffs filed the application

that resulted in the '471 patent, that is U.S. Patent Application number 08/192093, the '093 application. The '093 application was a continuation-in-part of both the '413 application and another application. The '471 patent was issued on September 4, 2001 and standing alone would expire on September 4, 2018.

On February 4, 1994, plaintiffs also filed the application that led to the '272 patent, that is U.S. Patent Application number 08/192102, the '102 application. This application was also a continuation-in-part of the same application as the '471 patent. The '272 patent was issued on August 12, 1997 and expired on August 12, 2014.

On October 18, 1994, plaintiffs filed the application that led to the '195 patent, U.S. Patent Application number. 08/324799. This application was also a continuation-in-part of the -- well, this application was a continuation-in-part of the '083 application and two other applications. The '195 patent was issued on December 16, 1997 and expired on December 16, 2014.

01:12 The '471 patent claims a group of chimeric antibodies. Claims 1, 3, 5, 6 and 7, encompass the infliximab antibody, which is also called cA2. The '471 does not claim infliximab specifically, however the specification describes the infliximab antibody and refers to it as a preferred embodiment of the invention. The '195 patent claims methods of using cA2,

meaning infliximab, for treatment of rheumatoid arthritis. In particular, Claim 6 recites, "a method of treating rheumatoid arthritis in a human comprising administering to the human an effective TNF-inhibiting amount of chimeric anti-TNF antibody, cA2."

The '272 patent claims methods of using cA2, again meaning infliximab, for treatment of TNFa-mediated Crohn's disease. In particular, Claim 7 recites "a method of treating TNFa-mediated Crohn's disease in a human comprising administering to the human an effective TNF-inhibiting amount of chimeric anti-TNF antibody, cA2."

The relevant law concerning obviousness double patenting includes the following: "A later claim that is not patentably distinct, i.e., is obvious over or anticipated by an earlier claim is invalid for obviousness-type double patenting," as the Federal Circuit says in *Sun*, 611 F.3d at 1185.

"There are two justifications for obviousness-type double patenting," as the Federal Circuit said in *Hubbell*, 709 F.3d, 1145. The first justification "prevents unjustfied time-wise extension of the right to exclude granted by a patent no matter how the extension is brought about."

"The obviousness-type double patenting analysis involves two steps. First, the Court construes the claims in the earlier patent and the claims in the later patent and determines the differences.

"Second, the Court determines whether those differences render the claims patentably distinct." That's *Sun*, again, at 1385.

"A later claim that is not patentably distinct from, i.e., is obvious over or anticipated by an earlier claim is invalid for obviousness-type double patenting."

Defendants have framed the three questions of particular importance to this motion. They are: One, are the plaintiffs entitled to the statutory 35 U.S.C. Section 121 safe harbor? I find plaintiffs are not.

The second question is, if the Section 121 safe harbor is not applicable, which test for obviousness applies, the one-way test or the two-way test? I find that the one-way test applies in this case.

Third is the issue of whether, if the two-way test applies, it is proper to consider the '471 patent specification when conducting the analysis. And if so, whether the '471 patent is invalid. I find that it is proper to consider the specification, and when that is done, the '471 patent is invalid.

With regard to the safe harbor issue, 35 U.S. Code Section 121 establishes a safe harbor against double patenting invalidation for certain patents issued for patent applications that are divisional of earlier applications, as the Federal Circuit said in *Pfizer*, 518 F.3d at 1359. The statute provides

that "if two or more independent and distinct inventions are claimed in one application, the director may require the application to be restricted to one of the inventions. If the other invention is made the subject of a divisional application, which complies with the requirements of Section 121, it shall be entitled to the benefit of the filing date of the original application. A patent issuing on an application with respect to which a requirement for a restriction under this section has been made, or on an application filed as a result of such a requirement, shall not be used as a reference either in the Patent and Trademark Office or in the courts against a divisional application or against the original application or any patent issued on either of them if the divisional application is filed before the issuance of the patent on the other application. The validity of a patent shall not be questioned for failure of the director to require the application to be restricted to one invention."

The Federal Circuit applies "a strict test for application of Section 121, given the potential windfall, a patent term extension could provide to a patentee," the Federal Circuit said in *Searle*, 790 F.3d 1349 at 1354. Section 121 safe harbor applies only to patents where the application was labeled "divisional." After filing an original parent application, an applicant can file subsequent continuing applications that claim the priority date of the parent pursuant to 37 CFR

Section 1.78.

There are multiple types of subsequent applications that may be filed, including divisional applications, and continuations-in-part, or CIPs.

In *Pfizer*, the Federal Circuit considered the question of whether the Section 121 safe harbor applied to a patent resulting from an application labeled "continuation-in-part" and not "divisional." The District Court held that the '068 patent at issue in that case, which was filed as a CIP, could not be invalidated for double patenting by the '165 patent in that case because both "derived from applications filed in response to the restriction requirement made in the common parent application." The Federal Circuit reversed, holding that, "The protection afforded by Section 121 to applications or patents issued therefrom filed as a result of a restriction requirement is limited to divisional applications." That's *Pfizer* at 1362.

In reaching its conclusion, the Federal Circuit in *Pfizer* rejected the plaintiffs' argument here that "the terms 'divisional' and 'continuation-in-part' are merely labels used for administrative convenience" and "the term 'divisional application' as it is used in Section 121, refers broadly to any type of continuing application filed as a result of a restriction, regardless of whether it is labeled by the PTO or for administrative purposes as a divisional, a continuation or

a CIP." That's *Pfizer* at 1360.

The Court reviewed the legislative history of Section 121, noting that prior to its passage, "no protection was afforded to patent applications filed as a result of a restriction requirement, and such applications were often rejected or held invalid on double patenting grounds." That's *Pfizer* at 1360 to 61.

The Court explained that Congress recognized the inequity of requiring an applicant to split out inventions into separate applications and then using those separate applications that the PTO compelled to invalidate resulting patents. That's also *Pfizer* at 1361.

However, the Court noted that "there is no suggestion in the legislative history of Section 121 that the safe harbor provision was or needed to be directed at anything but divisional applications" and that "the difference between deliberate applications and CIPs was well known at the time of the 1952 Patent Act." That's at 1361 to 62.

The Federal Circuit concluded that "if the drafters wanted to include CIPs within the protection afforded by Section 121, they could have easily done so." In *Pfizer*, the Court concluded, at 1362, "the protection afforded by Section 121 to applications or patents issued therefrom filed as a result of a restriction requirement is limited to divisional applications."

In *Amgen*, 580 F.3d 1340 at 53, the Federal Circuit stated

that, "We are persuaded by the reasoning in *Pfizer* that the Section 121 safe harbor provision does not protect continuation applications or patents descending from only continuation applications. The statute on its face applies only to divisional applications, and a continuation application, like a continuation-in-part application, is not a divisional application."

In *Amgen* at 1354, the Court was addressing a continuation application. It says, "We decline to construe 'divisional application' in Section 121 to encompass Amgen's properly filed, proper designated continuation application." As I said earlier in the instant case, continuation-in-part applications are involved.

In *Amgen*, the Federal Circuit noted that "unlike a continuation-in-part application, a continuation application can satisfy the definition of a divisional application in MPEP, Section 201.06. This is because continuation-in-part applications add subject matter not disclosed in the earlier application, whereas continuation in divisional applications are limited to subject matter disclosed in the earlier application. This distinction, however, does not justify departing from a strict application on the plain language of Section 121 which affords its benefits to divisional applications."

So *Amgen* was addressing a factual situation that would

have been more favorable to the plaintiffs here and nevertheless held that the plain language of Section 121 affords its benefits to divisional applications only.

I understand that the PTO is now reexamining the '471 patent, and I'm advised that it has allowed an amendment to label that application a divisional. However, this action is not final and therefore is not effective pursuant to 37 CFR Section 1.530(k). Moreover, as I understand it, if any such amendment becomes final, it will not be one filed before the issuance of the other relevant patents as required by Section 121.

This was addressed in *Searle*, 790 F.3d 1349 at 1354 to 55. There the Federal Circuit wrote, with regard to the patents at issue there, "The '113 application for the '068 patent cannot be a divisional of the '594 application despite being designated as such in the reissue patent because it contains new matter that was not present in the '594 application. Simply deleting that new matter from the reissued patent does not retroactively alter the nature of the '113 application because neither of the '068 patent applications is a division of the original '594 application. The Section 121 safe harbor does not apply to the RE '048 patents."

It is undisputed that the application leading to the '471 patent was filed as a continuation-in-part and not denominated a divisional. Plaintiff argues that this Court has the

discretion to consider whether to deem the '471 patent application divisional. They argue that the Court, in *Amgen*, 1354, leaves the possibility open when it stated that, "Amgen has not presented us with any persuasive reason as to why we should deem the '178 and '179 continuing application divisional applications for purposes of Section 121."

They argue that the alleged facts in plaintiffs' presentation on this motion, those in Exhibit D, slide 61, mandate deeming the '471 patent application as divisional. However, in *Amgen*, the Federal Circuit addressed and rejected a comparable argument at page 1354 and 53. Essentially, *Pfizer*, *Amgen* and *Searle,* or in those cases the Federal Circuit set out a bright line rule: only patents emerging from applications filed as divisional may benefit from the Section 121 safe harbor. I do not have the discretion to deem the '471 patent application divisional, therefore, the '471 patent is not protected by the Section 121 safe harbor.

The next issue concerns whether the usual one-way test for determining obviousness double patenting or the unusual two-way test should apply. The defendants argue for the one-way test. The plaintiffs argue for the two-way test.

"The one-way test analyzes whether the claims of a challenged patent are obvious in light of the claims of an earlier-referenced patent," as the Federal Circuit said in *Hubbell*, 709 F.3d at 1149. Under the two-way test, the Court

conducts the one-way analysis and also analyzes "whether the reference patent claims are obvious over the challenged patent claims." That's *Hubbell* at 1149. "Thus, when the two-way test applies, some claims may be allowed that would have been rejected under the one-way test," as the Federal Circuit described in *Berg* at 1432.

"The two-way test is a narrow exception to the general rule of the one-way test," the Federal Circuit said in *Hubbell* at 1149. "The two-way test is appropriate only in the unusual circumstance where the PTO is solely responsible for the delay in causing the second filed application to issue prior to the first," which is also *Hubbell* at 1149. The "typical scenario" where the two-way test is applied is "when a later-filed improvement patent issues before an earlier-filed invention," as explained in *Berg* at 1434.

"Basic and improvement patents should not be penalized by the rate of progress of the applications through the PTO, a matter over which the applicant does not have complete control," the Federal Circuit said in *Braat*, 937 F.2d at 593.

In this case, it is undisputed that on February 2, 1993, plaintiffs filed the '413 application, which is one of the parent applications of the '471, '195 and the '272 patents. On February 4, 1994, plaintiffs filed the application that resulted in the '471 patent, the '093 application. The '471 patent was issued on September 4, 2001 and standing alone would

expire on September 4, 2018.

Also on February 4, 1994, plaintiffs filed the application that led to the '272 patent, the '102 application. The '272 patent was issued on August 12, 1997 and expired on August 12, 2014. On October 18, 1994, plaintiffs filed the application that led to the '195 patent. That is application '799. The '195 patent was issued on December 16, 1997, and it expired on December 16, 2014. Plaintiffs' counsel confirmed in the hearing on this motion that plaintiffs do not contest, or at
01:37 least do not contest for present purposes, that the '471 patent is obvious for double patenting over the '195 patent or the '272 patent if the one-way test applies.

I find that this conclusion is correct. I also find that the one-way test is the applicable test. The '471 and '272 patents were filed on the same day. The Federal Circuit in *Berg* stated that, "The two-way test is appropriate only in the unusual circumstance that the PTO was solely responsible for the delay in causing the second filed patent application to issue prior to the first." That's *Berg* at 1437.

01:37 The Federal Circuit has reiterated this standard in *Hubbell*, 709 F.3d at 1149, and *Fallaux*, 546 F.3d 1313 at 1316. Here, the PTO did not decide the applications in reverse order of filing because the applications of the '471 patent and the '272 patent were filed the same day. Therefore, the language of *Berg*, *Hubbell* and *Fallaux* indicates that the two-way

patenting test is not available, with regard to those two patents at least.

The United States Patent and Trademark Office's Manual of Patent Examining Procedures, the MPEP, comes to the same conclusion. It states, "If both applications are filed on the same day, only a one-way determination of distinctness is needed in resolving the issue of double patenting." That's in the MPEP, Section 804.II(B)(2)(b). The MPEP cites in re: *Berg* for this proposition, and plaintiffs argue that *Berg* does not

01:39 control. I actually agree that *Berg* does not control because it didn't decide this issue, although the issue was presented in *Berg*.

In *Berg*, the inventor chose to file two applications simultaneously for inventions the Federal Circuit found could have been included in a single application. That's at page 1433. The Federal Circuit found that, "Simultaneously filing two separate applications that could have been filed in one application disqualifies the applicant from the two-way test." That's at 1435.

01:40 The Federal Circuit neither accepted nor rejected the PTO's other reason for finding the two-way test inapplicable the fact that the applications at issue were filed on the same day.

In the instant case, Janssen originally filed all of its claims in a single application in 1993. The PTO compelled it

to file multiple applications, two of which were filed on the same day, February 4, 1994, as continuation-in-part applications. The third CIP application was filed in October 1994.

In *Berg* at 1435 to 36, the Federal Circuit addressed what should be done to avoid obviousness double patenting if the PTO determines that claims in a single application belong in multiple applications. It said that, "The inventor should file one or more divisional applications, which will be protected by the Section 121 safe harbor provision." Janssen did not do this, although I recognize it was acting before *Berg* was decided.

Although the facts are different, that is, in *Berg* the applicant could have filed a single application, in Janssen, this case, the applicant could not, I find it is reasonable to conclude, as the Court did in *Berg* at 1235, that the applicant here should be "viewed as having taken the calculated risk that by simultaneously filing two applications." The applications for the '471 genus patent and the application for the '272 patent covering the method for treating Crohn's disease on the same day in 1994, "it might gain the advantage of a quickly issued narrow patent and also the advantage of a broader application which took longer to issue as a patent but consequently had a later expiration date."

Here, the '272 patent with a method for treating Crohn's

disease issued in 1997. The broader '471 genus patent issued in 2001.

Janssen began getting patent protection in 1997 under the '272. If the obviousness double patenting doctrine is not applied here, in effect, Janssen's right to exclude the public from practicing the '272 would be extended to 2018.

As the Federal Circuit wrote in *Hubbell* at 1145, and as I said earlier, the first justification for obviousness double patenting is "to prevent unjustified time-wise extension of the right to exclude granted by a patent no matter how the extension is brought about." It is appropriate to apply this principle to find, as the PTO would, according to its manual, that the usual one-way test applies. As indicated earlier, plaintiff concedes for present purposes that the '471 is invalid for obviousness double patenting under this test.

In the interest of completeness, I am also going to address the issue of how the two-way obviousness test would operate if it were applicable in this case. As I said earlier, "the two-way test is appropriate only in the unusual circumstance that the PTO was solely responsible for the delay in causing the second-filed application to issue prior to the first." The Federal Circuit said that in *Berg*, 140 F.3d at 1437. It reiterated it in *Hubbell*, 709 F.3d at 1149, and in *Fallaux*, 546 F.3d at 1360.

The Federal Circuit wrote in *Hubbell* at 1145,

"Obviousness-type double patenting is a question of law we review de novo. We review the Board's factual findings for substantial evidence." In the context of this case, as the statement indicates, there may be a genuine dispute concerning whether the PTO was solely responsible for the delay that caused the earlier-filed '471 to issue after the later-filed '195.

In *Engineered Products*, 225 F. Supp. 2d. 1069 at 1089, Judge Mark Bennett in the Eastern District of Iowa recognized there could be such a case. In such a case, if it were material, I would have a jury decide the disputed fact. I would then use that factual finding in deciding whether the two-way test applies, that is, whether the PTO was solely responsible for the delay that caused the later filed application to result in a patent issued first.

As I said during the argument, this is how this judge at least handles issues relating to qualified immunity, which is ultimately a legal question but may depend on the facts.

The plaintiff contends there are disputed facts and therefore summary judgment is not proper with regard to the two-way test. However, even assuming without finding that whether the PTO was solely responsible for the delay is in genuine dispute, that fact is not material to the outcome of this motion for summary judgment because the plaintiffs would fail the two-way test.

If the two-way test were applicable, one of the primary issues disputed by the parties is whether the Court may consider the '471 patent specification when analyzing the second prong of the two-way test, whether the claims of the '195 and '272 patents are obvious in light of the '471 patent.

I find that it is proper for the Court to consider the '471 patent specification. "It is well settled that the Court may look to a reference patent's disclosure of utility to determine the question of obviousness," the Federal Circuit said in *AbbVie*, 764 F.3d 1366 at 1381. "The Federal Circuit has repeatedly approved examination of the disclosed utility of the invention claimed in an earlier patent to address the question of obviousness," the Court said at 1382.

The Federal Circuit stated in *Sun*, 611 F.3d at 1387, that "The specification's disclosure may be used to determine whether a claim merely defines an obvious variation of what is earlier disclosed and claimed to learn the meaning of the claim terms and to interpret the coverage of a claim. Where a patent features a claim directed to a compound, a Court must consider the specification because the disclosed uses of the compound affect the scope of the claim for obviousness-type double patenting purposes."

Here, it is undisputed that the '471 patent is to a compound. Therefore it is appropriate to consider the specification. Plaintiffs argue that *Sun* is restricted to

circumstances where the claims of the patent do not refer to a utility of the invention. However, the Federal Circuit in *Sun*, 611 F.3d at 1385 to 86, rejected essentially the same argument, stating that "The holding of *Geneva* and *Pfizer* that a claim to a method using a composition is not patentably distinct from an earlier claim to the identical composition in a patent disclosing the identical use extends to any and all such uses disclosed in the specification of the earlier patent." Therefore it is proper for this Court to consider the '471 patent specification.

Here, the '471 patent specification discloses the utility of the claim genus of compounds and the infliximab antibody in particular for treating Crohn's disease and rheumatoid arthritis. The '471 patent describes the infliximab antibody in the specification, for example, at column 20, even including it as a preferred embodiment of the invention in column 14, line 57, to column 15, line 8.

The '471 patent specification discusses the utility of this class of compounds in treating various ailments, including rheumatoid arthritis and the TNFa-mediated Crohn's disease. It describes two studies where the infliximab antibody specifically was used to treat rheumatoid arthritis and Crohn's disease. Therefore, the '471 patent specification expressly describes the method of using the infliximab antibody to treat Crohn's disease and rheumatoid arthritis that are claims in the

'195 and '272 patents.

The Federal Circuit stated in *Geneva*, 349 F.3d at 1385 to 86, that, "A claim to a method of using a composition is not patentably distinct from an earlier claim to the identical composition in a patent disclosing the identical use." Therefore, the '195 and '272 patents are obvious in light of the '471 patent under the second prong of the two-way obviousness test. As both prongs of the two-way obviousness test are satisfied, Claims 1, 3, 5, 6, and 7 of the '471 patent are invalid for obviousness-type double patenting.

Plaintiffs argue that, even if the Court considers the '471 patent specification, disputed facts bar the Court from concluding that the '195, '272 claims are obvious in light of the '471 patent. They argue that the '471 patent claims recited genus of antibodies, not the infliximab antibody in particular. Meanwhile, the '195 and '272 patents recite methods for using an infliximab antibody itself. They argue that it is a disputed fact whether the methods of using a specific species in the genus are rendered obvious in light of the genus.

This argument ignores the fact that the '471 patent specification expressly describes using the infliximab antibody itself to treat Crohn's disease in rheumatoid arthritis. The '471 does not limit its discussion to the more general question of using the genus to treat these diseases.

So for those reasons I find that the '471 patent is not protected by the Section 121 safe harbor provision. The one-way test is the test that should be applied. And as plaintiffs concede, for present purposes, the '471 patent fails that test. I find that even if the two-way test were applied, the '471 patent would be invalid for obviousness double patenting. Therefore, the defendants' motion for summary judgment on this issue is hereby allowed.

All right. It's five minutes of 2:00. We're going to take a five-minute break. And is the plaintiff still asking that the trial be expedited?

MR. DISKANT: Not exactly. I could make my pitch in a minute.

THE COURT: Here. What are you going to be asking for?

MR. DISKANT: I'm going to be explaining why we made the motion in the first place, which is that things are urgent, as Your Honor has said. I recognize that counsel have trial schedules. I'm not trying to interfere with your trial schedules. That left only a week after Thanksgiving. I heard Your Honor wasn't inclined to do that, so we're asking for a trial in February, which is when Your Honor --

THE COURT: That's when it's scheduled.

MR. DISKANT: That's correct. We'd like a date certain. I've talked to Mr. Hurst about the timing of that.

CERTIFICATE OF FILING AND SERVICE

I, Robyn Cocho, hereby certify pursuant to Fed. R. App. P. 25(d) that, on January 26, 2017 the foregoing Brief for Plaintiffs-Appellants was filed through the CM/ECF system and served electronically on parties in the case:

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